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13. ABSTRACT (Maximum 200 words)

This project focused on the initial evaluation of potassium ferrate (VI) as an effective decontaminant for CWAs. The technical objectives were:. (1) Quantify the extent, if any, to which a ferrate (VI)-based decontamination of HD and VX generated toxic degradation products. (2) Measure the thermal stability of potassium ferrate (VI) at 71°C, in accordance with AR 70-38, under isothermal and cyclic temperature conditions. (3)Determine the decontamination yields of potassium ferrate (VI) on HD and VX.

Potassium ferrate (VI) demonstrated greater than 99% destruction efficiencies for decontaminating HD and VX at ambient conditions without the formation of toxic degradation products. Under isothermal and cyclic storage conditions of 71°C, potassium ferrate (VI) retained 90% and 93% of its initial purity after 98 and 82 days, respectively. At the conditions tested, the agents appear to have mostly converted to LCNNTOs or fully mineralized.

With these results, all of the project deliverables were met successfully. Coupled with its known efficient biocidal properties and probable low toxicity, corrosion, hazard, and environmental impact features, ferrate appears to possess the needed properties for a broad-spectrum, low-cost, general purpose, widely distributed chemical-biological decontamination (CBD) reagent.

These results justify taking the next steps in developing this unique reagent into a widely distributed product for CBD.

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FINAL REPORT

Solid Thermally Stable Peroxide-Equivalent Chemical Warfare Agent Decontamination Reagent

ARO Project No.: W911NF-05-C-0051
BAA W911NF-04-R-0006 "Topic Area II.B.4.a"

to

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October 22, 2005

EXECUTIVE SUMMARY

Executive Summary

This project focused on the initial evaluation of potassium ferrate(VI) as an effective decontaminant for chemical warfare agents. The technical objectives were to:

1. Quantify the extent, if any, to which a ferrate(VI)-based decontamination of HD and VX generated toxic degradation products.
2. Measure the thermal stability of potassium ferrate(VI) at 71°C, in accordance with AR 70-38, under isothermal and cyclic temperature conditions.
3. Determine the decontamination yields of potassium ferrate(VI) on HD and VX.

Potassium ferrate(VI) demonstrated greater than 99% destruction efficiencies for decontaminating HD and VX at ambient conditions without the formation of toxic degradation products. Under isothermal and cyclic storage conditions of 71°C, potassium ferrate(VI) retained 90% and 93% of its initial purity after 98 and 82 days, respectively.

Three sets of tests were performed on HD and one test was completed on VX. All four test sets were run in triplicate. In addition to potassium ferrate(VI), the ferrate formulations included a phase transfer catalyst to enhance the solubility of ferrate(VI) with non-polar agents. The three HD test conditions were selected to evaluate the effect of pH and of ferrate(VI)-to-HD mass ratio. Run 1 was performed at conditions providing a final pH near 10.5 and with a ferrate(VI)-to-HD ratio of 22.5:1. Run 2 evaluated the effect of operating near a neutral pH of 7 with the same 22.5:1 ferrate(VI)-to-HD ratio. In Run 3, buffered at a pH of 7, ferrate(VI) was limited to a mass ratio of 2.7:1, forcing an incomplete reaction to enhance the presence of intermediate HD degradation products. Run 3 results helped develop the ferrate(VI) mass balanced decon reaction scheme for HD (Section 4.2.4).

Run 4 measured the destruction yield of VX and quantified key degradation products. The VX testing was conducted at a pH near 7-9 and a ferrate to VX mass ratio of 45:1. To quantify the destruction yield and key degradation products, chemical analyses were performed using GC, GC-MS, and LC-MS-MS. Chemical agent work was performed at Battelle's Hazardous Materials Research Laboratory, an ISO 9000-2001 registered chemical surety facility.

At a final pH near 10.5 and a ferrate(VI)-to-HD mass ratio of 22.5:1, 99.1% of HD was destroyed to the point where none of the targeted degradation products nor any other significant organic compounds could be detected by full-scan LC-MS or GC-MS. The pH 10.5-buffered aqueous reference with the phase-transfer catalyst and the unbuffered water control yielded reductions in HD of 89% and 88%, respectively. The final pH in Run 1 increased to 12.4, indicating the pH 10.5 buffer capacity was exceeded. Even at this higher pH, no toxic degradation products were detected. Reducing the pH to 7 in Run 2 and maintaining the 22.5 ferrate(VI)-to-HD mass ratio lowered the HD destruction to 80% versus 44% for the pH 7-buffered aqueous control. Hence, ferrate decontamination is still very effective at mild pH and that higher ferrate use amounts than the level used here appear to be needed at neutral pH. Severely limiting the amount of ferrate in the third HD test was designed to enable HD degradation products to survive in appreciable amounts. In this case HD degradation of 59% was still found with this limited 2.7:1 ferrate (VI)-to-HD mass ratio was expectedly low but significantly higher than the 29% of HD removed by the pH 7-buffered control. The primary degradation products observed at pH 7 and limited ferrate availability were the desirable divinyl sulfone at 278 µg/mL and thiodiglycol at 24 µg/mL. Therefore, these two products appear to be the intermediates formed as HD is decontaminated by ferrate ultimately becoming low carbon number non-toxic organics (LCNNTOs) and/or mineralized.

Treating VX with ferrate at pH ~7 and a 45:1 mass ratio yielded a 99.99% destruction versus a reduction of 66% and 59% for the pH 10.5-buffered reference and unbuffered water controls. No toxic VX degradation products were detected with ethyl methylphosphonic acid (EMPA) being the primary product at a 28.5% yield, along with 0.2% diisopropylamino ethanol being detected, lower molecular

weight, non-toxic organics and mineralized products comprising 71.3% of the VX degradation products. In contrast, both the pH-buffer/ phase transfer catalyst (PTC) reference and the unbuffered water controls generated measurable quantities of S-diisoprpoylaminoethy methyl phosphonic acid (EA2192), a highly toxic, water soluble, and stable degradation product of conventional VX-decontamination methods.

At the conditions tested, the agents appear to have mostly converted to LCNNTOs or fully mineralized. The use of reagent at substoichiometric levels relative to mineralization indicates that the decontamination reactions proceed through nontoxic intermediates. Another tentative conclusion offered is that toxic products that normally form with conventional hydrolysis treatments by water and alkaline pH, in the absence of ferrate, either do not have time to form when fast-reacting ferrate is present, or are destroyed rapidly by ferrate if they do form as intermediates. Most likely both modes of decontamination occur.

Potassium ferrate (VI) was found to be thermally stable at AR 70-38 test conditions for long periods, at least 98 days at 71°C, and at least 82 days cycling daily from 23°C to 71°C, regardless whether the potassium ferrate is pure (90-95%) or of only moderate purity (70-80% technical grade). In these tests, losses with time varied slightly with test vials ranging from <1% to 10% (±3%) loss after the test periods given.

All tests were conducted at relatively mild-reaction conditions of ambient temperature, mild pH, and ambient pressure. Although not yet optimized, reagent use rates were comparable to conventional decontamination reagents such as DF-200.

With these results, all of the project deliverables were met successfully. Coupled with its known efficient biocidal properties and probable low toxicity, corrosion, hazard, and environmental impact features, ferrate appears to possess the needed properties for a broad-spectrum, low-cost, general purpose, widely distributed chemical-biological decontamination (CBD) reagent.

Hence, these results justify taking the next steps in developing this unique reagent into a widely distributed product for CBD and also, due to ferrate's versatile

chemistry, for toxic industrial chemical (TIC) and non-traditional agent (NTA) decontamination applications.

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ACRONYMS

ALS	Automatic liquid sampler
APCI	Atmospheric Pressure Chemical Ionization
AR	Army regulations
ARO	Army Research Office
BCO	Battelle Columbus Operations
CA	Chemical agent
CB	Chemical biological
CBD	Chemical biological decontamination
CoC	Chain of Custody
CSM	Chemical surety material
CWA	Chemical warfare agent
DEA	Diethylamine
D	Decontamination (as part of an acronym)
DI	Deionized (as referring to water purity)
DIPAE	2-N,N-diisopropylaminoethanol
DoD	Department of Defense
DTRA	Defense Threat Reduction Agency
$DVSO_2$	Divinyl sulfone
ECBC	Edgewood
EMPA	Ethoxy methylphosphonic acid
Ferrate	Ferrate, $FeO_4^{=}$, Potassium Ferrate(VI), ferrate
FS	Full scan spectrum collection, as in mass spectrum
FT	Ferrate Sample
GC	Gas chromatography
GC-MS	Gas chromatography with mass spectrometric detection
HPLC	High pressure liquid chromatography
HML	Hazardous Materials Laboratory (located at West Jefferson, Ohio)
HMRC	Hazardous Materials Research Center (located at West Jefferson, Ohio)
HTH	High test hypochlorite
ICV	Instrument Calibration and Verification
ID	Identification

IDL	Instrument detection limit
ISO	International Standards Organization
LC-MS	Liquid chromatograph with mass spectrometric detection
LC-ESI-MS-MS	Liquid-chromatographic electrospray-ionization mass-spectrometry
LCNNTO	Low carbon number non-toxic organics
LRB	Laboratory record book
MDL	Method Detection Limit
MSDS	Material safety data sheet
NA	Not Applicable
NC	Negative control
ND	Not Detected
POD	Proof of decontamination
PTC	Phase transfer catalyst (in this report, normally the quaternized ammonium ion compound N-methyl N,N,N-trioctylammonium sulfate, technical grade)
PVOH	Polyvinyl Alcohol
QA/QC	Quality assurance/quality control
RCRA	Resource Conservation and Recovery Act
SOP	Standard operating procedure
SOW	Statement of work
Std Dev	Standard deviation
TDG	Thio diglycol (potential product of HD decontamination)
TDGO2	Thio diglycol sulfone (potential product of HD decontamination)
TEA	Triethylamine
TIC	Toxic industrial chemical (referring to chemicals that are a threat similar to CWAs due to the combination of certain chemical and physical properties)
TICA	Total ion chromatogram analysis
TPCS	Test performance control sheets
UHP	Ultra-high purity
UV/VIS	Ultraviolet/visible light

1 OBJECTIVES

The Army Research Office (ARO) has determined that a better CWA decontamination reagent is required for widespread use. Current reagents suffer from one or more of the following problems: too hazardous, not sufficiently storage stable, too corrosive, and/or form toxic products from CWAs. In addition, a need exists for more decontamination capacity per unit volume/weight of reagent, for a solid decontamination reagent, and for ease in cleanup after use.

This project is designed to determine certain critical properties of solid ferrate (hereafter referred to as ferrate) for decontamination applications:

- Determine stability of potassium ferrate to withstand thermal storage at 71°C, i.e., essentially as defined by AR 70-38, Sec. II, Table 2-2 (Storage and Transit Conditions).

- Determine if toxic products form when ferrate is used to decontaminate HD or VX; more specifically, identify the yields and products formed during the decontamination of VX and HD using ferrate.

- Demonstrate that ferrate is able to destroy HD and VX substantially at room temperature conditions for which the products from the point above were determined.

2 INTRODUCTION AND BACKGROUND

2.1 Description of Need

The hazards and excessive corrosivity of decontamination reagents such as chlorine-based chemicals (e.g., bleach, chlorine dioxide, and high test hypochlorite (HTH)) and caustic chelating amines (e.g., DS2) are well established. DF-200, a proprietary blend of organic and inorganic materials developed at Sandia Corporation, is the current most preferred decontamination reagent (Tadros, 2003). The patent suggests that the formulation includes a cationic surfactant solubilizing agent (quaternary ammonium surfactant, e.g., cetyltrimethyl ammonium bromide, benzalkonium chloride, polymeric quaternary ammonium compounds, etc.) at 0.1-10%, an emulsifying agent, water soluble polymer (e.g., polyvinyl alcohol (PVOH), guar gum, and/or other synthetic polymers) at 0-10%, long chain fatty alcohol (C_{10-16}) at 0-1%, a corrosion inhibitor (e.g. diethylamine (DEA), triethylamine (TEA), organo nitrite, or N,N-dibenzylamine), a catalyst (e.g., iodosobenzoate and copper amine complexes), and an optional "oxidizer/ nucleophile." The oxidizer can be a peroxide, urea hydrogen peroxide, hydroperoxycarbonate, oximates, alkoxides, aryloxides, aldehydes, peroxymonosulfate, Fenton's reagent, and hypochlorite, including blends that can form other inorganic or organic peroxides *in situ* from more readily available materials such as H_2O_2. DF-200 can also be formulated to provide a substantial foaming action. Since the Client already has substantial knowledge of the proprietary materials of DF-200 and its CWAs decontamination products, comparing DF-200 and ferrate decontamination products is possible, at least in part, as a result of the work performed in this project.

Although water, sunlight, aging, and detergents are useful in certain decontamination activities, these mild reagents are slow to react, do an incomplete job of decontamination, or form very stable toxic products (e.g., EA 2192 in the case of VX, as summarized in Section 4.3). Mild decontamination reagents also can spread toxic chemicals around the cleanup area and cause them to transfer to other sites such as protective garments, absorbed into materials, onto floors, into sewers, and transported to waste water treatment facilities. Hence, Department of Defense

(DoD)-driven efforts by the ARO, Defense Threat Reduction Agency (DTRA), and others are underway to identify a more efficacious reagent(s). The benefits of a low hazard, noncorrosive, decontamination-effective, storage-stable, and environmentally compatible reagent are substantial and include a much less hazardous cleanup environment; a much broader range of uses regarding people, vehicles, and facilities; and rapid payback of cost due to reduced personnel and facility downtime and/or the avoided cost of replacement of equipment and facilities.

2.2 Peroxides as CWA Decontamination Reagents

Most peroxides are environmentally friendly materials when decomposing to harmless products, i.e., water, oxygen gas (O_2), simple carboxylic acids (for organo peroxides such as alkyl hydroperoxides), or inorganic salts (for inorganic peroxides such as monopersulfate, perborate, magnesium peroxide, etc.). All peroxides are inherently unstable and can undergo readily catalyzed self electron exchange redox reactions (disproportionation) resulting in premature and often rapid decomposition of the peroxide to the above benign products and loss of strength. Disproportionation of peroxides is catalyzed readily by many materials such as dissolved metal ions, surfaces, dust particles, alkalinity, and temperature. Peroxides are sensitive to thermal runaway decomposition if very concentrated since, at least for hydrogen peroxide, the disproportionation reaction is exothermic with the heat driving off water, which further concentrates the H_2O_2 and causes the reaction to proceed even faster. Lastly, peroxide reactions often decompose autocatalytically, i.e., self catalyzing. If pure, certain peroxides are stable at room temperature and, in fact, require a catalyst (normally a transition metal ion capable of oxidation state cycling) to facilitate rapid disproportionation or to cause homolytic cleavage to generate free radical intermediates.

On the other hand, peroxides decompose CWAs without formation of toxic products (Tillman and Kaplan, 1994). However, after these exhaustive studies, no peroxide, including organic, inorganic and polymeric versions, was found to possess the required thermal storage stability. Long-term storage is a critical requirement since an extensive worldwide decontamination reagent pipeline needs to be filled as

the locations of CWA attacks are uncertain and each demands a prompt response. Also, the need exists for higher agent/reagent decontamination weight ratios. DF-200, for example, is used at reagent: agent ratios in decontamination applications of 50:1. Solid decontamination reagents are desired for storage, handling, and having less inert ingredients per weight or volume.

2.3 Ferrate (VI) Reagent for Commercial Water Purification and for General Decontamination

Ferrate (VI) (simplified to just "ferrate" in this report) was selected some years ago by Battelle and others (Carr, J. D., 1975; Goff and Murmann, 1971; Waite and Gray, 1998) as a general-purpose, low-hazard, stable solid, environmentally acceptable, and efficacious broad-spectrum water purification reagent for commercial use to simultaneously replace chlorine-based oxidants, ferric or alum coagulants, which contain a high percentage of dead weight salt, and lime or caustic soda addition. In these small tests, ferrate has been proven to supply this needed reactivity. To date, commercial quantities do not exist. Recently, however, Battelle had identified a scaleable, low-cost ferrate production process [Monzyk et. al., 2004].

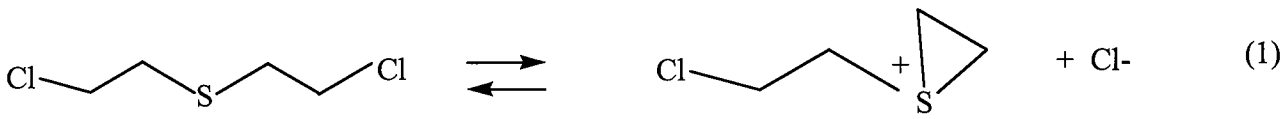
Ferrate has been studied less than peroxides because of its unavailability. Even so, the above academic researchers have surmised that its mode of oxidation is by oxygen atom transfer reagent and is not a free radical generator. Such reactivity is desirable as the probability of forming toxic products by random free radical activity is reduced, and the probability of good reaction control is enhanced. In addition, ferrate solid has been reported to have high thermal stability, >> 100°C though the basis for this conclusion had not been published. With Battelle's new ferrate production process and CWA decontamination testing and analytical capability, it is possible to determine precisely the following:

- Thermal storage stability of ferrate salts and formulations thereof
- Decontamination products and yields that are produced using ferrate as the decontamination reagent
- Procedures for performing broad spectrum chemical-biological defense (CBD) with ferrate.

2.4 Pertinent HD Chemistry and Selection of Ferrate Decontamination Test Conditions

Researchers, led by DoD laboratories, have become aware that attempts to decontaminate CWAs can lead to troublesome stable and still highly toxic products (Yang et al., 1986; 1192, 1997; Pellenbarg and Smiroldoz, 1986; Tillman and Kaplan, 1994). Hydrolysis, water content, and pH have been shown to be critical parameters to controlling reaction paths in decontamination chemistry towards nontoxic products and away from toxic residuals. A further objective and benefit of decontamination product design is to accomplish decontamination rapidly (<30 min and preferably <15 min), shown to be possible for ferrate in previous Battelle-funded testing (von Fahnestock, et al., DTRA poster presentation), and with the least amount of reagent. The role of each of these parameters arises from considering the prior literature.

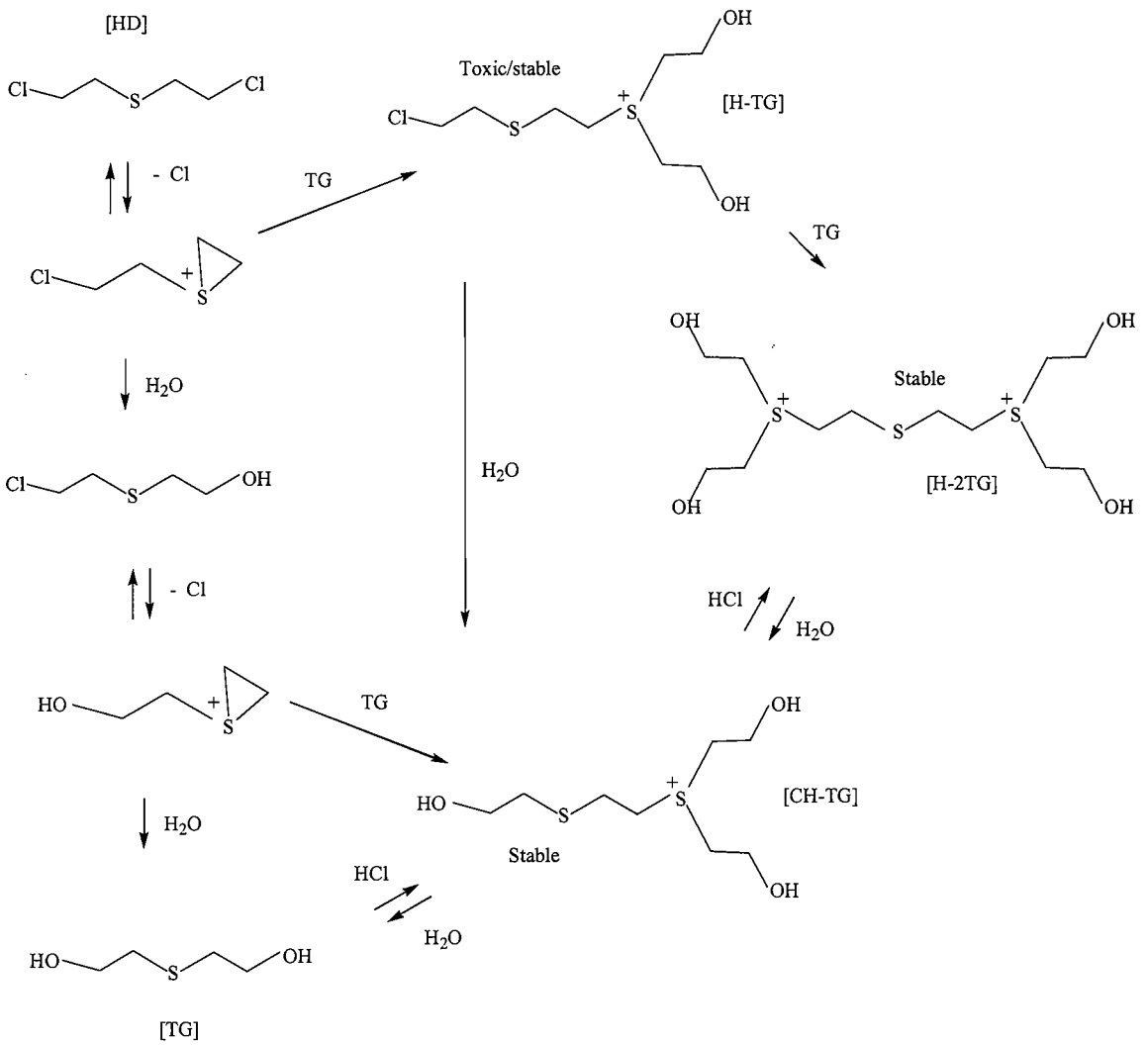
Certain reactions are independent of the above parameters, e.g., the equilibrium internal cyclization of HD (Reaction Scheme 1).



(1)

Reaction Scheme 1. HD Internal Self Cyclization in Water Resulting in Dehalogenation.

This cyclic intermediate can react to form stable compounds, some of which are still toxic (Reaction Scheme 2). This reaction supports the need to oxidize the thio group quickly in decontamination treatment. The tendency for HD to coalesce into droplets and films in aqueous environments, due to its low water solubility, promotes intermolecular reactions. Hence, HD decontamination chemistry must be capable of reacting rapidly across liquid phase boundaries.

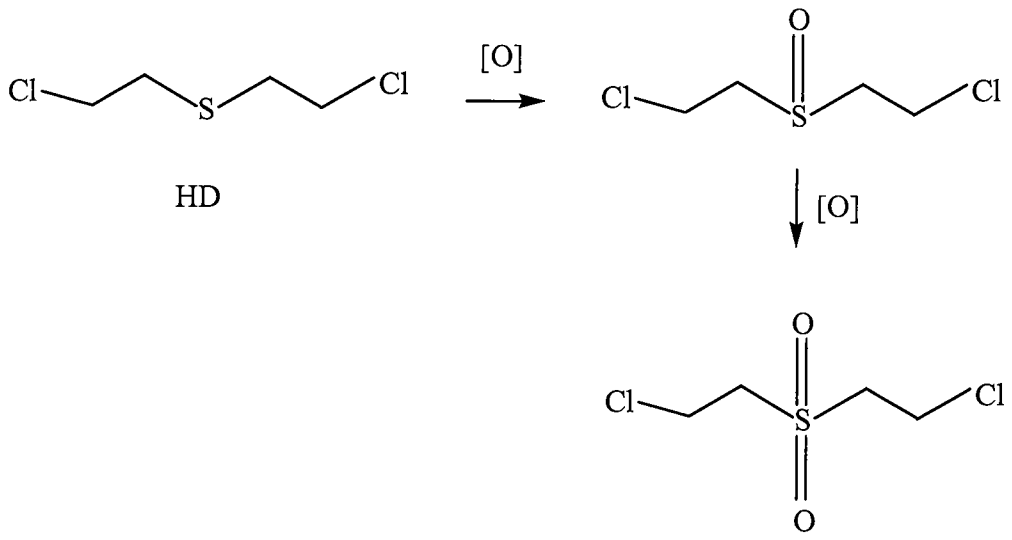


(2)

Reaction Scheme 2. HD Intramolecular and Intermolecular Reactions Leading to Formation of Toxic Products.

This mode of reactivity exemplifies the need for fast oxidation decontamination chemistry. This reaction scheme illustrates some of the thio intermediates of HD which lead to stable and sometimes toxic products.

With oxidants, a class that includes ferrate, the HD decontamination reaction course is projected to be much more favorable (Reaction Scheme 3).



(3)

Reaction Scheme 3. Illustration of Desired Low Reagent Weight Decontamination Reaction of HD Provided by Oxidation of the S-Atom.

Once the S-atom is oxidized, either to sulfoxide or sulfone, the agent rapidly and irreversibly is rendered nontoxic by fast and spontaneous hydrolysis at mild conditions to bis glycol nontoxic products, which readily form the desirable divinyl sulfone (not shown). The sulfoxide either is oxidized directly to the sulfone or can disproportionate to the sulfone and HD, which is quickly reoxidized.

In this case, the thio group is oxidized rapidly by O-atom transfer to nontoxic sulfoxide and/or very low toxicity sulfone products. If the sulfoxide/sulfone is produced quickly and at mild conditions, as could be possible with ferrate, then any hydrolysis results in the formation of nontoxic products, especially bis (2-hydroxyethyl) sulfone (or thio diglycol sulfone, $TDGSO_2$), the corresponding sulfoxide, and/or the half-hydrolysates mono (2-chloroethyl) derivatives of these oxidized HD products. More specific oxidation chemistry is provided in Section 4 based on the data collected during this project.

In such decontamination reactions, ferrate reaction is conveniently depicted as:

$$K_2FeO_4(s) + 3/2\ H_2O \rightarrow FeOOH + 3/2\ \text{“O”} + 2\ KOH \qquad (4)$$

Reaction Scheme 4. Illustration of Ferrate (VI) Oxidation Half-Reaction

Where the “O” designation indicates that O atoms are transferred to the compound being oxidized during the transition state of the decontamination reaction under

consideration. Through this test program, the HD decontamination reactions of ferrate were determined for the first time. A reasonable projection is Reaction Scheme 3, combined with Reaction 4, followed by hydrolysis in the same treatment using hydroxide ions generated in Reaction 4, but at a slower rate to give the overall Reaction 5 in the presence of moisture (balanced).

$$4K_2FeO_4(s) + 6H_2O + 3ClCH_2CH_2SCH_2CH_2Cl \rightarrow 4FeOOH + 2KOH + 6KCl + 3HOCH_2CH_2SO_2CH_2CH_2OH \quad (5)$$

Reaction Scheme 5. Projected HD Decontamination Chemistry by Ferrate

In the presence of orthophosphate buffer (Ferrate Decontamination Formulation No. 1.1), Reaction 6 would be expected for the pH range of 3-11.

$$4K_2FeO_4(s) + 4H_2O + 3ClCH_2CH_2SCH_2CH_2Cl + 2KH_2PO_4 \rightarrow 4FeOOH + 6KCl + 2K_2HPO_4 + 3HOCH_2CH_2SO_2CH_2CH_2OH \quad (6)$$

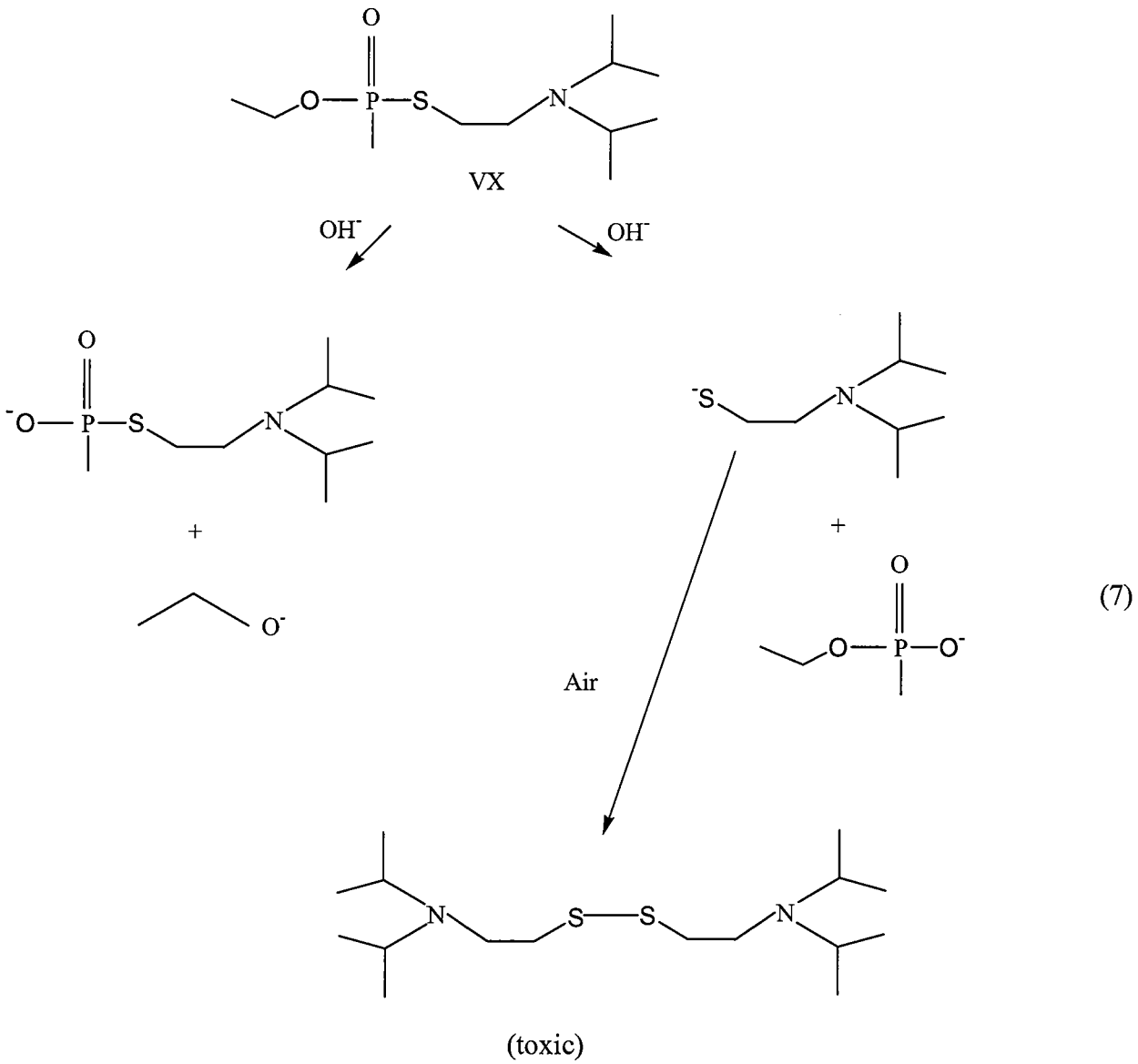
Reaction Scheme 6. Projected HD Decontamination Chemistry by Ferrate with Phosphate Buffer Present

This $TDGSO_2$ product could dehydrate readily to divinyl sulfone, another nontoxic product. Hence, the products of interest in the case of HD decontamination by ferrate are represented by Reactions 1 through 6 and divinyl sulfone, where both the sulfoxide and sulfone forms should be considered though the sulfoxide might be considered an intermediate due to its capability to disproportionate. All studies involving HD should include well-known problematic products found with other decontamination chemistries, such as caustic treatment, i.e. dithiane, divinyl sulfide, cyclic thio/oxy ethers, etc.

2.5 Pertinent VX Chemistry and Selection of Ferrate Decontamination Test Conditions

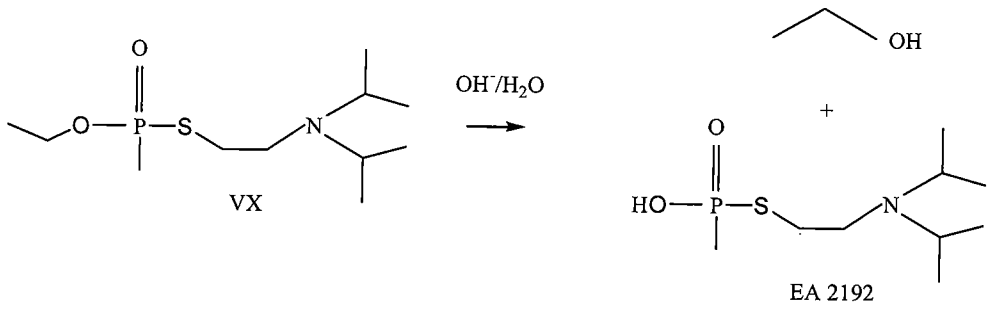
VX can form toxic products readily during decontamination operations, depending on conditions. Reaction Scheme 7 provides the well-established scheme for the parallel path hydrolysis reactions of VX at alkaline pH. Of most concern is

the hydrolysis of the ethoxy group, which leads to the well-characterized, stable, and water-soluble compound EA-2192 (Reaction Scheme 8). The hydrolysis release of the mercaptan can lead to a stable dithiol, but also to nontoxic ethyl methylphosphonate. Although avoiding EA 2192 formation, the free mercaptan produced by direct hydrolysis of the P-S bond can lead to formation of toxic metaphosphinates (Reaction Scheme 9). Therefore, rapid oxidation of the released mercaptan is desirable to prevent such toxic products.



(7)

Reaction Scheme 7. Alkaline Hydrolysis of VX Illustrating It's Well Known Dual Hydrolysis Competition Pathways of P-O vs P-S Bond Breakage.
The continued reactivity of the mercaptan intermediate also is illustrated, forming a disulfide.



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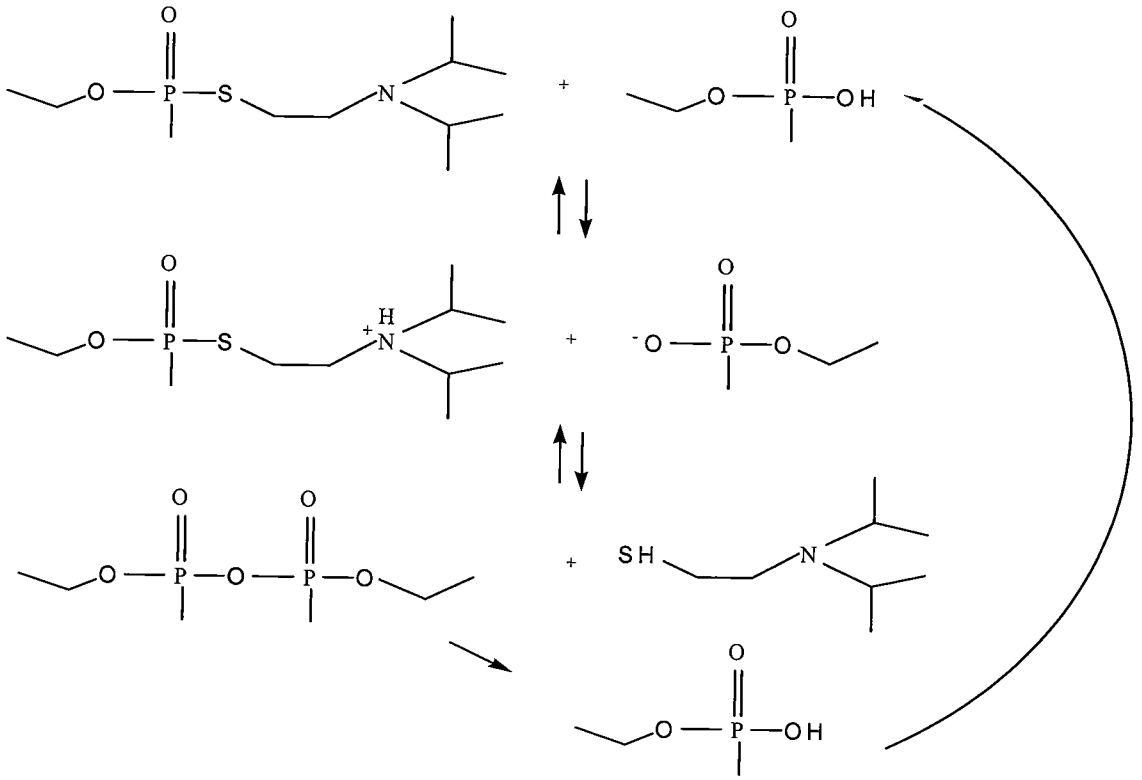
Reaction Scheme 8. Formation of Toxic VX Decontamination Product EA 2192

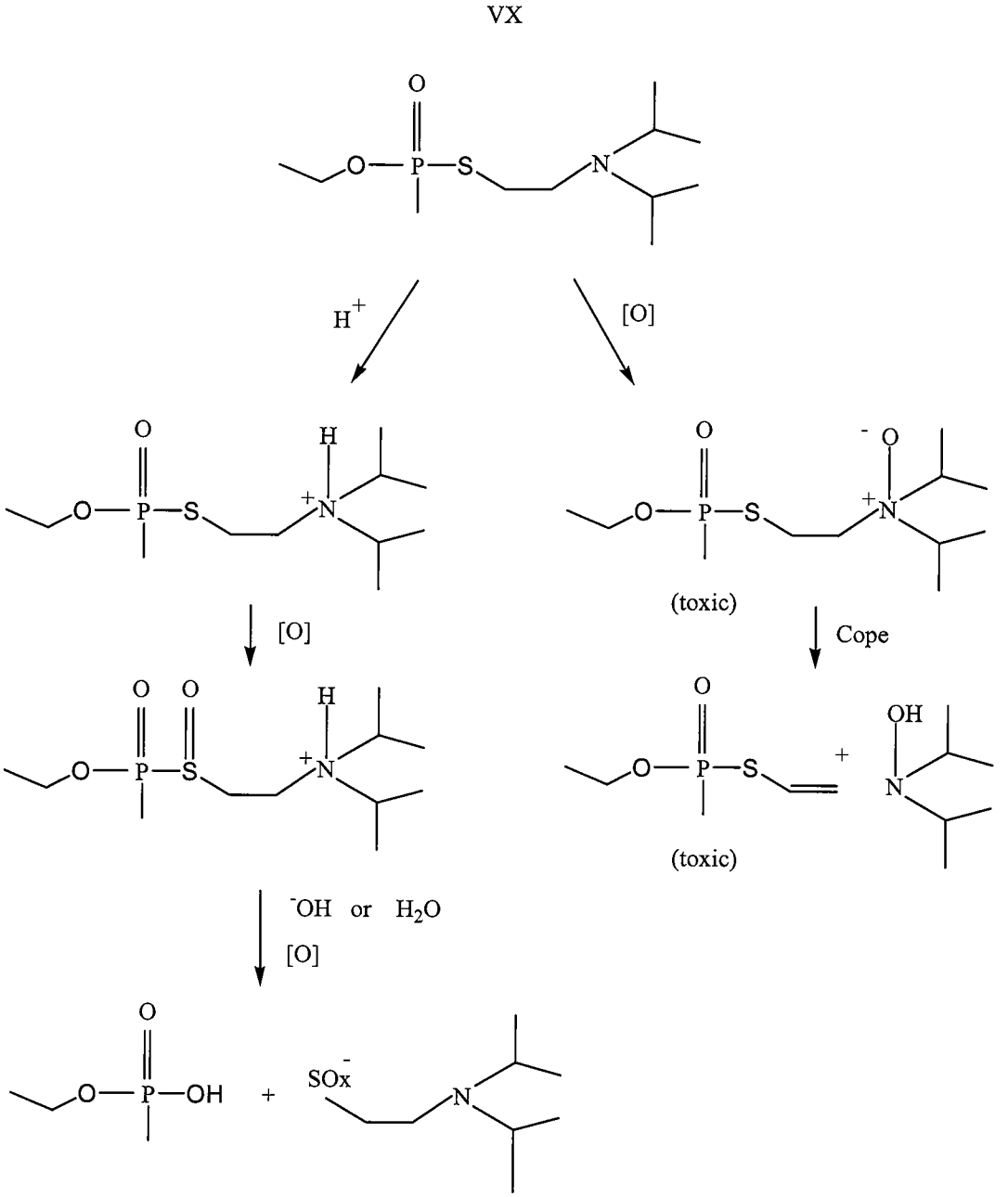


(9)

Reaction Scheme 9. Formation of Toxic Metaphosphonate from Mercaptan Hydrolysate Intermediate from VX.

An oxidative environment is needed to prevent formation of stable toxic products via mercaptans.

However, oxidants also form toxic products if used to decontamine VX at high pH (Reaction Scheme 10, right side of the reaction flow scheme).



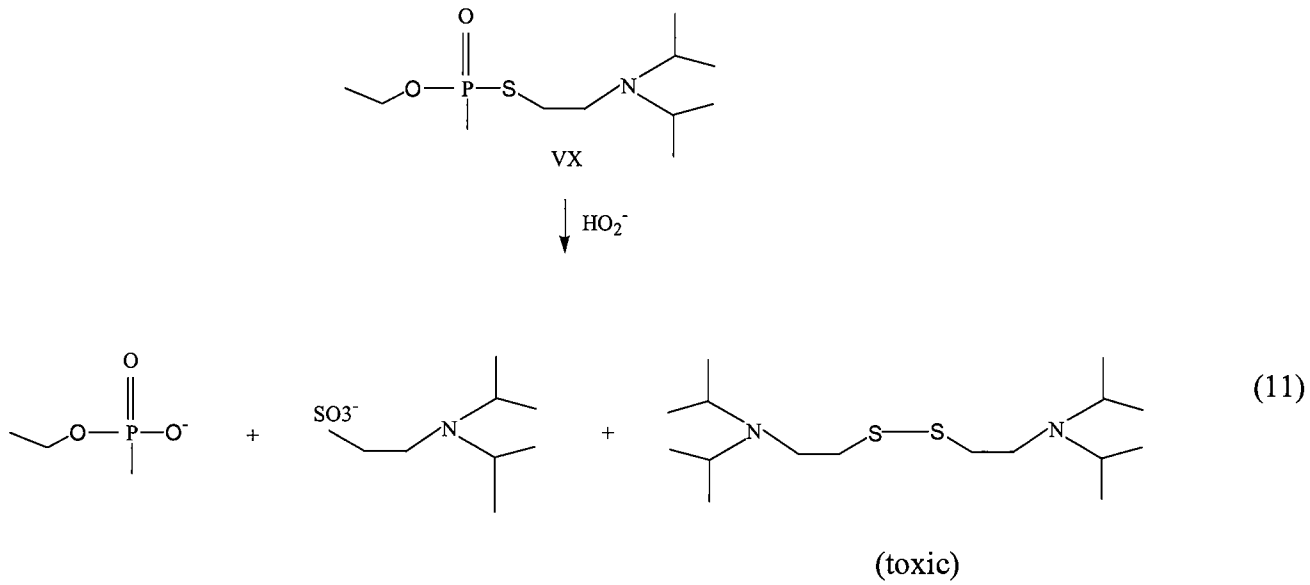
(10)

Reaction Scheme 10. Preferred VX Decontamination Chemistry Target (left sequence of reactions)

Protonation of the amine group is followed by fast oxidation of the S with fast P-S bond hydrolysis. Degree of final product phosphonic acid and amine protonation/deprotonation depends upon final pH of reaction mixture.

Toxic compounds, in this case, form through N→O bond formation by oxidation of the amine which occurs faster than S-oxidation, perhaps due to the delocalized thio

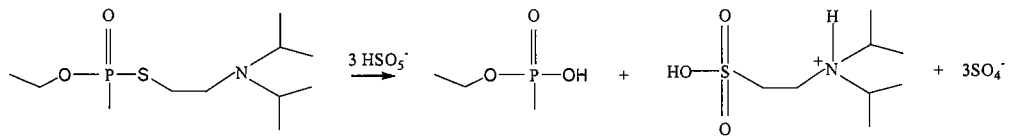
electrons into the phosphonate group and the availability of the localized lone pair of electrons of the trialkylamine N. These N→O compounds form toxic compounds directly or indirectly, as Reaction Scheme 10 illustrates. For example, hydrogen peroxide, H_2O_2, is a highly reactive oxidant at alkaline pH values where the HO_2^- species is formed. This species is a strong nucleophile, promotes the desirable P-S bond hydrolysis, and achieves the desired oxidation of the intermediate mercaptan (Reaction Scheme 11). However, competitive disulfide bond formation competes with the slow oxidation by H_2O_2 to form stable and toxic bis-(diisoppopylaminoethyl) disulfide (Reaction Scheme 7).



(11)

Reaction Scheme 11. Ionized Peroxide (HO_2^-) Nucleophilic Substitution, Oxidation and Hydrolysis of VX at High pH (due to High pKa of H_2O_2).

Due to slow reactivity of peroxide and high pH, toxic disulfide product also is formed. The high pH drives premature hydrolysis (Reaction Scheme 7) to release mercaptan, making possible oxidation formation of disulfide. Hence, pH<pKa (H_2O_2) is the preferred decontamination condition, except when slow reactivity of H_2O_2 results.



(12)

Reaction Scheme 12. Illustration of Most Preferred Peroxide-Based Decontamination Reaction for VX.

Oxidant is monopersulfate ion from Oxone, an acidic corrosive material (pH ~ 2). Products are nontoxic. Unfortunately, peroxides are not thermally stable due to ease of autooxidation and disproportionation reactions.

The VX decontamination reaction of choice is rapid oxidation at a pH where the amine is protonated. The pKa of the ammonium group, ~9, indicates that a pH of <9 is preferred and a pH <8 is most preferred. The desirable reaction sequence is given in Reaction Scheme 10 (left side set of reactions) where the initial pH is <8. When the final reaction pH is <8, the final nontoxic products are the salt of the methyl ethylphosphonate and the alkyl ammonium sulfonate zwitterion (not shown in Reaction Scheme 10).

For example, literature reviews showed that the inorganic peroxide monopersulfate, at low enough pH to avoid fast alkaline hydrolysis prior to oxidation, provides a desirable path (Reaction Scheme 12) (Tillman and Kaplan, 1994; Yang, et al, 1997).

For Reaction Scheme 12, the oxidant monopersulfate, HSO_5^-, is shown. This oxidant is available as the strongly acidic triple salt, Oxone ($2KHSO_5*KHSO_4*K_2SO_4$), which is about 4.7% active oxygen (49.5% potassium monopersulfate). Interestingly, the acid medium oxidation potential of monopersulfate is -1.44 V versus -2.2 V for ferrate; yet the persulfate is far more unstable thermally due to the long single bond of the peroxo group (-O--O-) which is easily rearranged for autooxidation and catalyzed disproportionation reactions. In Reaction Scheme 12, the sequence is that the acidic media first protonates the amine of VX, forming HVX^+, rendering the N unavailable for oxidation. Such reactions are instantaneous (only diffusion controlled). Then the monopersulfate can oxidize only the S. Interestingly, Tillman and Kaplan (1994) and Y.C. Yang (1997) found that with N protonation at least one toxic compound forms if the conditions involve a nonpolar solvent (Reaction Scheme 10, left side). On the other hand, the combination of oxidant, protonation of the amine, and the use of polar solvent (e.g., water) are key parameters for VX decontamination without toxic product formation.

The above information was used to identify VX decontamination conditions using ferrate, where a buffered pH of 7±1 and water addition was used. The projected balanced VX decontamination reaction using ferrate follows. First, essentially instant protonation by the buffer system forms an ion cluster:

$$EtOP(=O)(CH_3)SCH_2CH_2N(iPr)_2 + KH_2PO_4 \rightleftharpoons EtOP(=O)(CH_3)SCH_2CH_2NH^+(iPr)_2,HPO_4^=,K^+]$$
VX VXH$^+$ (13)

Reaction Scheme 13. Protic Equilibrium Involved from Basic VX Reaction with Monobasic Orthophosphate forming Zwitterionic Product

The protonation reaction is followed by fast oxidation of the S and clearage of the P-S bond to form EMPA and LIO that are both non-toxic:

$$[EtOP(=O)(CH_3)SCH_2CH_2NH^+(iPr)_2,HPO_4^=,K^+] + 2K_2FeO_4 + 3KH_2PO_4 \rightarrow$$
VXH$^+$

$$\rightarrow [EtOP(=O)(CH_3)OFe^{III}(OH)_2] + [K^+{}^-O_3SCH_2CH_2NH^+(iPr)_2,HPO_4^=,K^+] + FeOOH + 3K_2HPO_4 \quad (14)$$
Nontoxic Nontoxic

Reaction Scheme 14. Selective Oxidation of S over N by Ferrate at Mild pH

the sulfonate ester then to hydrolyzes rapidly to the nontoxic sulfonate ion salt. The ferric hydroxide product could be present as ferric phosphate with the same general results. The half reactions for the above reaction schemes involve water, but no net water is consumed or produced. Hence, since potassium ferrate is very water soluble, only small amounts of water appear necessary to enable the decontamination reaction of the ferrate ion and VX. This case appears to be same with other CWAs. At pH 6-8, the final ferric products are expected to be the phosphonate complex shown and/or ferric phosphate, $FePO_4$, based on using orthophosphate as the buffer, as is the case with Ferrate Formulation No. 1.

3 EXPERIMENTAL METHODS AND PROCEDURES

3.1 Description of Experimental Approach

The laboratory evaluation of ferrate for CWA decontamination was split into the following four tasks:

1. Test Plan generation
2. Preparation of potassium ferrate decontamination reagent formulations
3. Potassium ferrate Thermal Stability Testing
4. HD and VX decontamination testing
5. HD and VX decontamination reaction product analyses
6. Data reduction and analysis

The experimental approach was based on the statement of work (SOW) stipulation that this project was a product chemistry determination, and not product formulation refinement or product development. Therefore, product refinement and development were outside the scope of this project. The experimental approach was to maximize influence of the available literature by devising a limited number of quantitative tests consisting of two unique decontamination ferrate formulations with the purpose of identifying yields and products produced from using a ferrate reagent.

Previous preliminary range finding tests conducted by Battelle (von Fahnestock et al., 2004) identified an effective formula, Formulation 1, that displayed attractive agent decontamination activity against blister and nerve agents, notably HD, VX, GB, and GD. Formulation 1 consisted of a certain blend of potassium ferrate solid, quaternary amine phase transfer catalyst in the sulfate ion form, and orthophosphate buffer. In addition, some water could have enhanced the decontamination performance of Formulation 1 (for this study). Formulation 1 CWA decontamination literature and known ferrate chemistry of ferrate, was used to generate the two unique formulations (Ferrate Decontamination Reagent Formulation No. 1.1 and No. 1.2) for this current study. These two formulations were used to devise four individual decontamination runs (Table 3.1). Ferrate

Formulation No. 1.1 (FF1) and Ferrate Formulation No. 1.2 (FF2) are described further in Section 3.2.2.

Table 3.1. Decontamination Run Matrix

Run	Agent	Projected pH	Reagent/Agent Ratio	Ferrate Formulation
1	HD	10.5	Medium	FF1
2	HD	7	Medium	FF2
3	HD	10.5	Low	FF1
4	VX	7	Medium	FF2

3.2 Potassium Ferrate Decontamination Reagent

3.2.1 Potassium Ferrate Production Process

The ferrate ion was produced by a proprietary Battelle process "skid unit" located at Battelle Columbus, pilot facility 5-2-030 (Figure 3.1). The final product of this method was a wet filter cake consisting of approximately 8-12% ferrate salt. This filter cake was converted to technical grade potassium ferrate crystals (K_2FeO_4 TG) and used in thermal stability and decontamination formulation testing.

Conversion of the filter cake to K_2FeO_4 TG was achieved through a recrystallization protocol developed by Battelle and modified from the procedure described by Schreyer, et al (1953). The filter cake product was dissolved in dilute potassium hydroxide and filtered to remove insoluble iron oxide components. Solid potassium hydroxide then was added to the filtrate, driving the formation of the potassium ferrate salt. The solution was chilled to lower the solubility of potassium ferrate and then filtered. The potassium ferrate crystals obtained on the filter were washed with solvents to remove remaining potassium hydroxide and to aid in water removal. The dried potassium ferrate salt was stored in a vacuum desiccator to prevent atmospheric moisture from degrading the product. Each sample of K_2FeO_4 TG produced was then assayed for K_2FeO_4 content to ±2% absolute by a two-wavelength ultraviolet/visible (UV/VIS) colorimetric method (Smeltz, A. et al., 2002).

Potassium ferrate formulations were prepared in sample vials. Each vial was prepared gravimetrically using a Mettler AG-245 electronic balance (Serial No. 1115230066, calibrated 10/5/2004, due 10/5/2005). The calibration of the balance was checked daily using a standardized mass set (Battelle Metrology Laboratory, Serial No. C16244, calibrated 4/20/2005, due 4/20/2006).

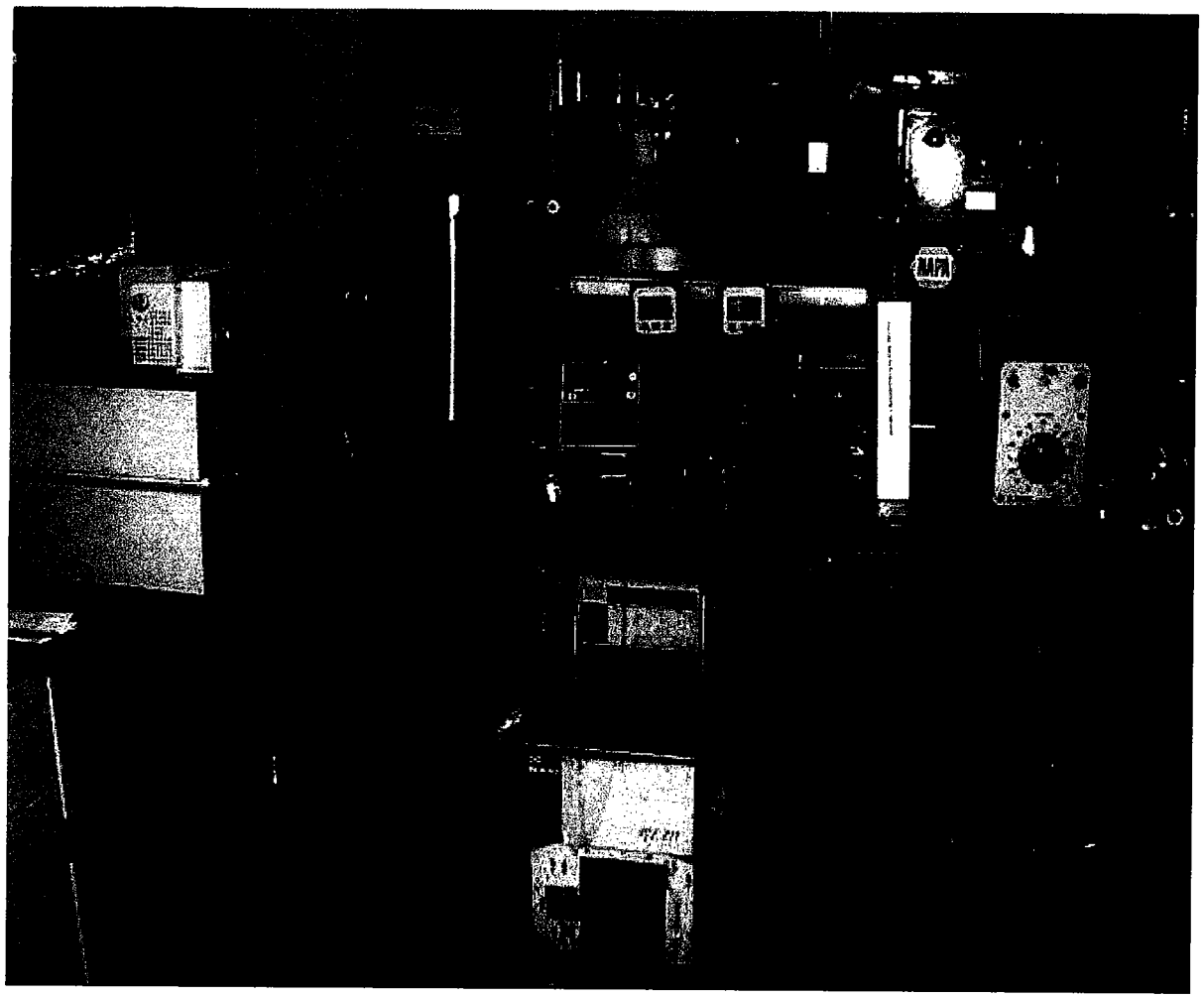


Figure 3.1. Battelle's Proprietary Ferrate Production Unit.

3.2.2 Potassium Ferrate Formulations

The two ferrate formulations (FF1 and FF2) were chosen to provide the most desirable decontaminated agent product mixture, i.e., nontoxic products and minimal

residual agent. Emphasis also was placed on a protocol that was practical for lab and, eventually, field use. Hence, both formulations used mild reaction conditions of ambient temperature, mild pH, ambient pressure, and reagent use rates the same or lower than conventional decontamination reagents such as DF 200.

A total of four decontamination runs in triplicate using ferrate solutions and water controls were conducted at room temperature. Run 1, Run 2, and Run 3 were carried out with HD; Run 4, with VX. Of the three HD runs, Run 1 and Run 2 were conducted at a ferrate to HD wt/wt ratio of 45:1 (Run 1 under basic conditions at a pH of 10.5 and Run 2 at pH 7.0). Run 3 was conducted at pH 7.0, but at a reduced ferrate to HD wt/wt ratio of 2.7:1. In order to achieve the ferrate to HD wt/wt ratio of 2.7:1 for Run 3, the HD level was increased to the maximum amount allowable within this study's standard operating procedure (Appendix A) for decontamination testing. The VX decontamination test (Run 4) was composed with a ferrate to VX wt/wt ratio of 45:1 and a pH 7.0. The pH of 7.0 is in the buffer range of orthophosphate and is low enough to avoid toxic reaction products of VX (refer to Section 1.0). All runs used only pre-thermally-treated potassium ferrate oxidant.

3.2.2.1 Run 1: Ferrate formulation 1 for treatment of HD with a large excess of potassium ferrate reagent at a pH of 10.5 using phase transfer catalyst (PTC).

To each of the three ferrate test (ferrate treatment) sample vials, 140 mg of potassium phosphate monobasic and 50 µL of aliquat-336 PTC were added. To each of the three reference (non-ferrate buffer) test sample vials, 31.5 mg of sodium bicarbonate, 27 µL of 10.0 N sodium hydroxide solution, and 50 µL of PTC were added. Three additional empty vials were used as water blanks (water reference). Finally, three vials were filled with approximately 278 mg of ~94% potassium ferrate crystals (260 mg of purity-adjusted material) and used in the testing of the ferrate treatments.

3.2.2.2 Run 2: Ferrate formulation 2 for treatment of HD with a large excess of potassium ferrate reagent at a pH of 7.0 using phase transfer catalyst.

To each of the three ferrate test sample vials, 750 mg of potassium phosphate monobasic and 50 µL of aliquat-336 PTC were added. To each of the three reference test sample vials, 372 mg of potassium phosphate monobasic, 624 mg of dipotassium hydrogen phosphate trihydrate and 50 µL of PTC were added. Three additional empty vials were reserved to be used as water blanks. Finally, three vials were filled with approximately 281 mg of ~93% potassium ferrate crystals (260 mg of purity-adjusted material). These three vials were mixed with the contents of the ferrate test sample vials during the HD decontamination testing.

3.2.2.3 Run 3: Decontamination formulation 1.1 for treatment of HD with an excess of potassium ferrate reagent at a pH of 10.5 using PTC.

To each of the three ferrate test sample vials, 140 mg of potassium phosphate monobasic and 50 µL of aliquat-336 PTC were added. To each of the three reference test sample vials, 31.5 mg of sodium bicarbonate, 27 µL of 10.0 N sodium hydroxide solution and 50 µL of PTC were added. Three additional empty vials were reserved to be used as water blanks. Finally, three vials were filled with approximately 281 mg of ~93% potassium ferrate crystals (260 mg of purity-adjusted material). These three vials were mixed with the contents of the ferrate test sample vials during the HD decontamination testing.

3.2.2.4 Run 4: Decontamination formulation 1.2 for treatment of VX with a large excess of potassium ferrate reagent at a pH of 7.0 using PTC.

To each of the three ferrate test sample vials, 750 mg of potassium phosphate monobasic and 50 µL of aliquat-336 PTC were added. To each of the three reference test sample vials, 372 mg of potassium phosphate monobasic, 624 mg of dipotassium hydrogen phosphate trihydrate and 50 µL of PTC were added. Three additional empty vials were reserved to be used as water blanks. Finally, three vials were filled with approximately 281 mg of ~93% potassium ferrate crystals (260 mg of purity-adjusted material). These three vials were mixed with the contents of the ferrate test sample vials during the VX decontamination testing.

All vials were shipped via Battelle courier to the Battelle Hazardous Materials Research Center (HMRC) along with a printed spreadsheet detailing the contents of each vial, and a Material Safety Data Sheet (MSDS) for each component. Agent testing was conducted at the HMRC.

3.3 K_2FeO_4 Thermal Stability Testing

The thermal stability of K_2FeO_4 was studied in accordance with AR 70-38 Sec. II, 2-4 (hot, dry climate), Table 2-2 (Storage and Transit Conditions) was studied. The required temperature profile appears below in Figure 3.2.

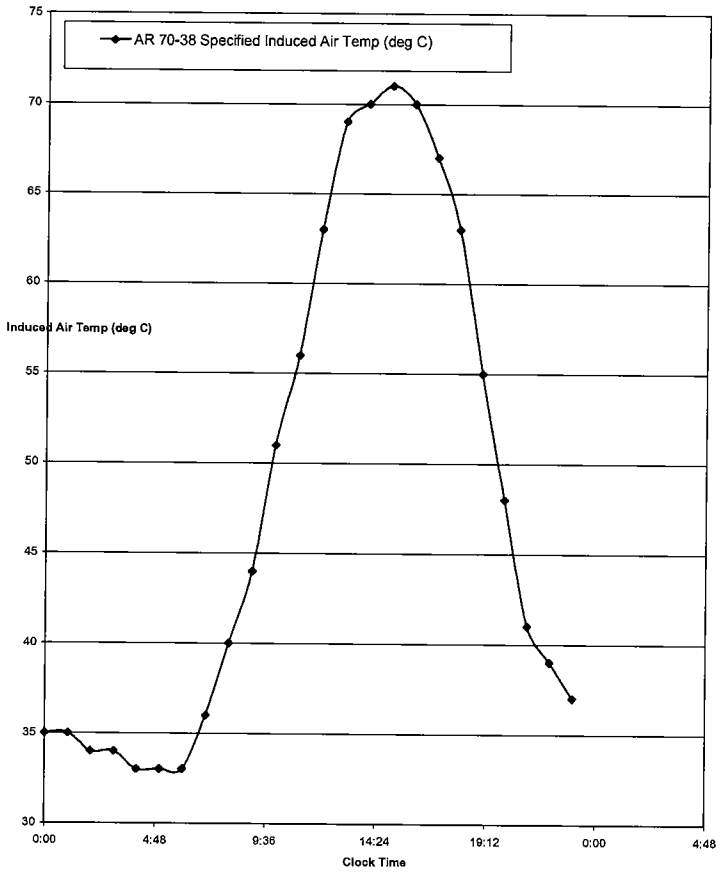


Figure 3.2. AR 70-38 Hot-Dry Temperature Cycle Requirement.

According to AR-38-70, testing at the highest temperature level (71°C) in lieu of temperature cycling is acceptable. The more demanding isothermal test

conditions were chosen for the preliminary viability assessment testing. In this series of tests, a Blue M Electric Oven, Model OV-490A-3 (S/N OV-11311, BMI No. N-00154, 120V Single Phase, 38-260°C), was used. The oven was set to maintain an air temperature of approximately 71.5°C. A mercury thermometer immersed in silicone oil was added to the oven for manual tracking of the temperature, and a HOBO model H08-002-02 external temperature logger (Onset Computer Corp., S/N 5948-9820) was used for automated temperature data acquisition. Four ~1.0 g samples of K_2FeO_4 TG were transferred to clean 12 mL glass vials with Teflon-lined caps; these samples with purity from ~75-93% were chosen specifically to represent a variety of initial purity values. Before closure, the headspace of each vial briefly was purged (~20 sec at ~250 cm^3 (STP)) with ultra-high purity (UHP) argon gas to minimize trapped moisture. The vials were placed into the oven on June 15, 2005 at approximately 1200 hr. A log sheet was placed on the exterior of the oven to track the removal and return of the samples. Photographs of the exterior and interior of the oven appear below in Figure 3.3 and 3.4, respectively.

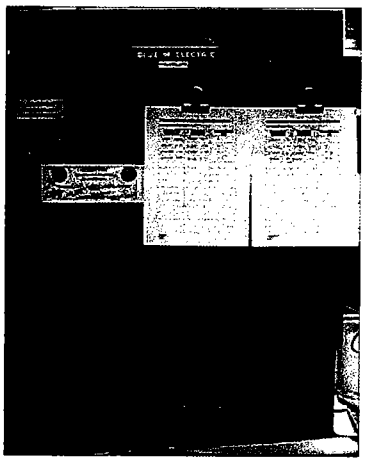


Figure 3.3. Isothermal Oven Exterior.

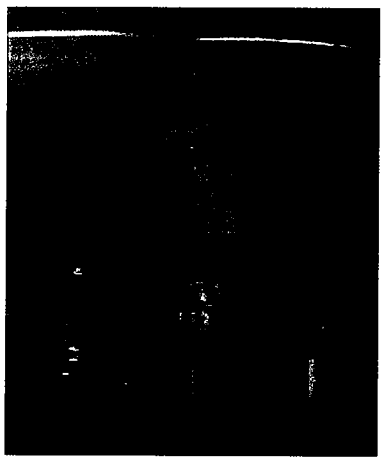


Figure 3.4. Isothermal Oven Interior.

The samples were removed from the oven at 1, 2, 5, 7, 12, 27, 48, and 98 days. The purity of each sample was determined by the technique described in Section 3.2.1. Each vial was assayed three times at each sampling point in order to calculate standard deviation and provide an estimate of precision. When returning

the vials to the isothermal oven, the headspace of each vial was purged briefly with UHP argon, as before.

The cycling temperature profile specified in AR 70-38 also was studied. An oven (Fisher Scientific model No. 48) was outfitted with a programmable temperature controller (Omega Engineering model CN3251) and a small fan in order to provide air circulation. Temperature data acquisition was performed using a HOBO data logger (Onset Computer Corp., model HTEA -39+123°C, serial number 168638). The temperature controller was programmed to meet the specifications of AR 70-38 Sec. II, Table 2-2 (Storage and Transit Conditions). Specifically, the oven was programmed to remain above 66°C for at least 5 hrs and to reach a peak temperature of 71°C for not more than 1 hr. The programmed parameters appear below in Table 3.2.

Table 3.2. Cycling Oven Programmed Parameters

Clock Time	Programmed Induced Air Temperature (°C)
0:00	35
6:30	35
12:30	66
15:00	71
17:30	66
23:30	35

The resulting temperature cycle for a typical 3-day period appears in Figure 3.5.

A vial of ~94% K_2FeO_4 TG with a Teflon-lined septa was placed in the oven on 1 July 2005 at approximately 1700 hr. A log sheet was placed on the exterior of the oven to track the removal and return of the sample. Photographs of the exterior and interior of the oven appear in Figures 3.6 and 3.7, respectively.

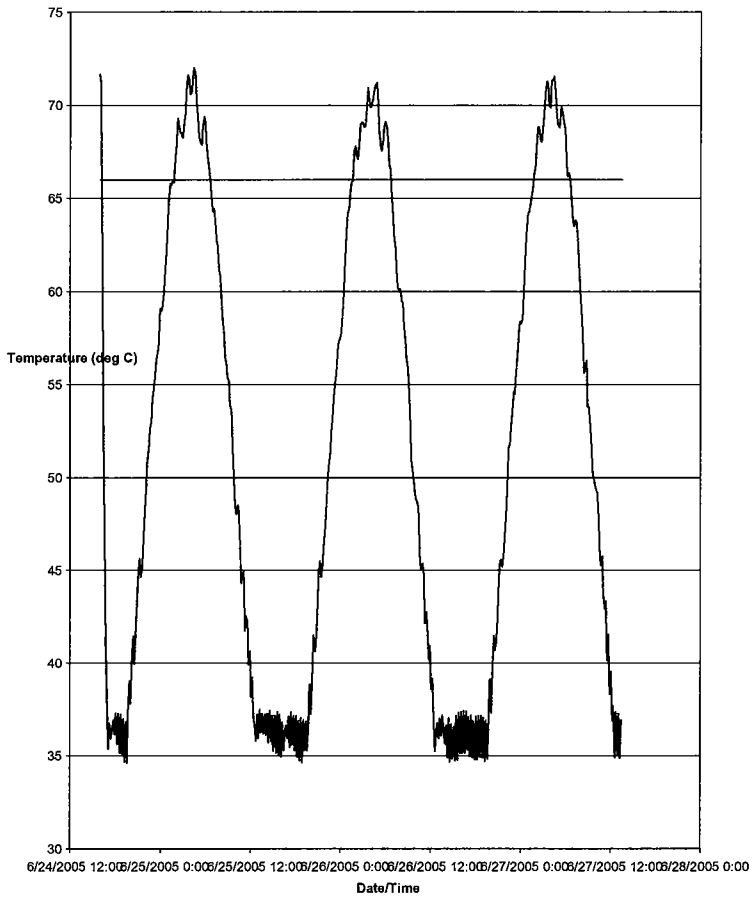


Figure 3.5. Cycling Oven Profile

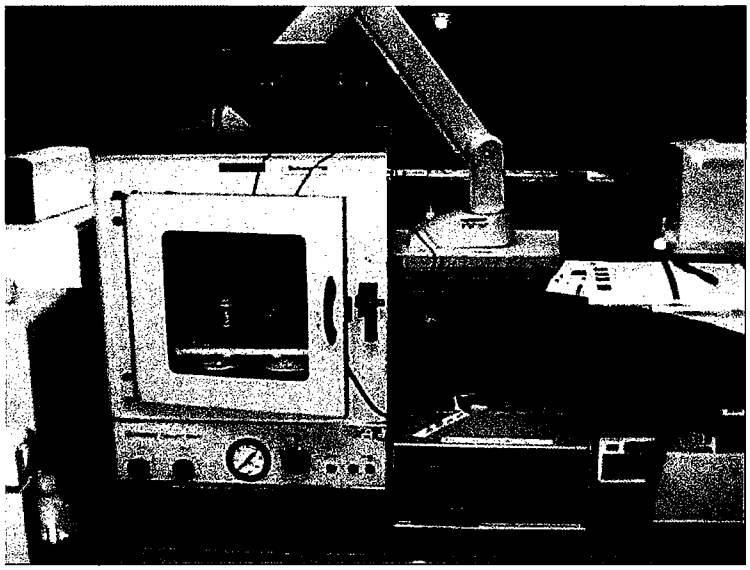


Figure 3.6. Cycling Oven Exterior with Ancillary Equipment.

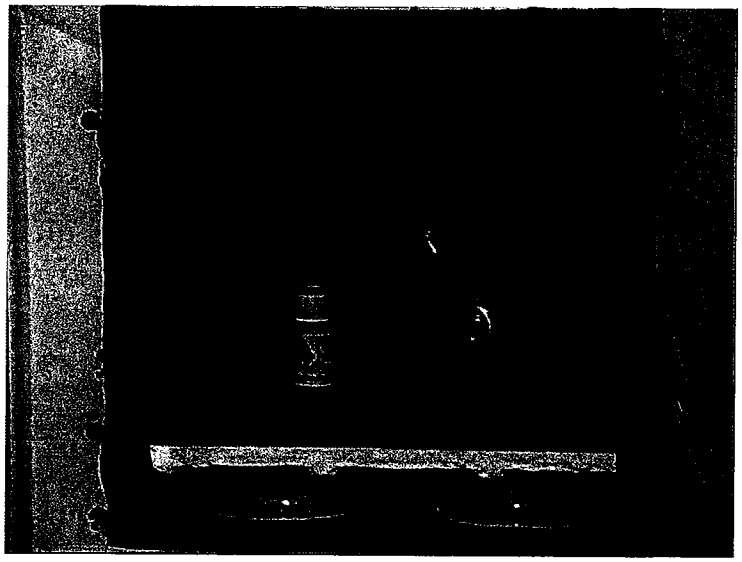


Figure 3.7. Cycling Oven Interior.

The sample was removed from the oven on day 11, day 32, and day 82. The purity of the sample was determined by the technique described in Section 3.1.1. The vial was assayed three times at each sampling point.

3.4 Decontamination Testing

3.4.1 Test Facility

The decontamination testing was carried out at Battelle's Hazardous Materials Research Center (HMRC), which operates under a bailment agreement with Edgewood Chemical and Biological Center (ECBC) The Underwriters Laboratory, Inc. certified the HMRC in accordance with International Standards Organization (ISO) 9001-2000.

3.4.2 Test Materials

The CAs HD and VX used in this study were on hand at the HMRC. The purity of each agent was determined before testing was initiated. The HD (Lot 8658) had a purity of 90.9%. The VX (Lot 8667) had a measured purity of 94.2%. The

agents HD and VX were selected because of their hydrophobic characteristics. The determination was made that these two agents represented the worst-case scenario and allowed the evaluation of PTC effectiveness in the ferrate formulation.

3.4.3 Test Equipment

The primary test equipment needed for decontamination testing was a hand-motion shaker as shown in Figure 3.8. The hand-motion shaker was used to ensure proper mixing of the samples and was set up in an approved fume hood.

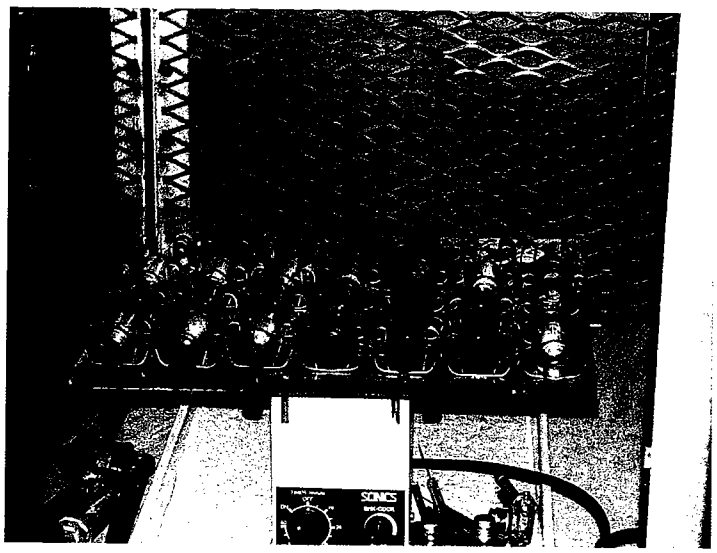


Figure 3.8. Hand-Motion Shaker.

3.4.4 Safety Considerations

For this study, a specific SOP was prepared (HMRC SOP-X-147-01, see Appendix A). Other SOPs already developed covered all additional procedures performed, including chemical surety materiel (CSM) handling, decontamination, disposal, evacuation, and emergency response. All technical and support personnel for decontamination testing were trained in the requisite procedures to ensure the safe handling of hazardous and toxic substances. In addition, decontamination testing did not begin until the approved test plan was received, use of agent was authorized by ECBC, and a safety dry run had been completed.

3.4.5 Test Procedure

The HMRC SOP-X-147-01 details the material preparation, test set-up methods, and sampling decontamination test methods. Therefore, only a general description is provided in this section.

Upon receipt of the ferrate components from the Battelle Columbus Operations (BCO), samples were prepared in 20 mL scintillation vials. Preparation included adding reagents and agent to each vial in rapid sequence, vortexing the test mixture for 10 seconds and then covering and mixing on a hand motion shaker for 60 min (Figures 3.8, 3.9 and 3.10). Next, the entire content of each HD sample was extracted with isooctane; a portion of the VX sample was extracted with isooctane, with the remainder kept in the aqueous phase (Figure 3.11). The organic phase of the HD and VX then was aliquoted into gas chromatograph (GC) vials; half of the vials were used for gas chromatogram-mass spectrometry (GC-MS) analysis and the other half was archived in a freezer. In addition, the aqueous phase of both the HD and VX samples was placed into 20 mL vials and shipped to BCO. Once the aliquot transfer was completed, the Chain-of-Custody (CoC) form and the GC vials were relinquished to the responsible analyst in the analytical laboratory, initiating a sequence of steps described in Section 3.4.7.

Figure 3.9. Example of a Ferrate Treatment Sample Before Shaking.
Purple color is due to presence of ferrate ion, $FeO_4^{=}$, and is visual assurance that viable decontamination reagent is being used and is present for the requisite minimum time required.

Note: The ten drops of HD required for Run 1 and 2 were accomplished by dispensing ten individual drops from a single 50 µl syringe, equipped with a stopper. Each drop was 1 µl ± 0.05 µl, based on the results of the spike controls. The 85 drops of HD required for Run 3 were accomplished by dispensing 50 individual drops from a 50 µl syringe, refilling the syringe and dispensing an additional 35 individual drops from the same 50 µl syringe. The six drops required for Run 4 were accomplished by dispensing six individual drops from a single 50 µl syringe.

The number of agent drops used for decontamination testing was recorded in Test Performance Control Sheets (TPCS) and noted in the Laboratory Record Book (LRB). In addition, the TPCS tracked test conditions such as the sample identification (ID), humidity, and temperature.

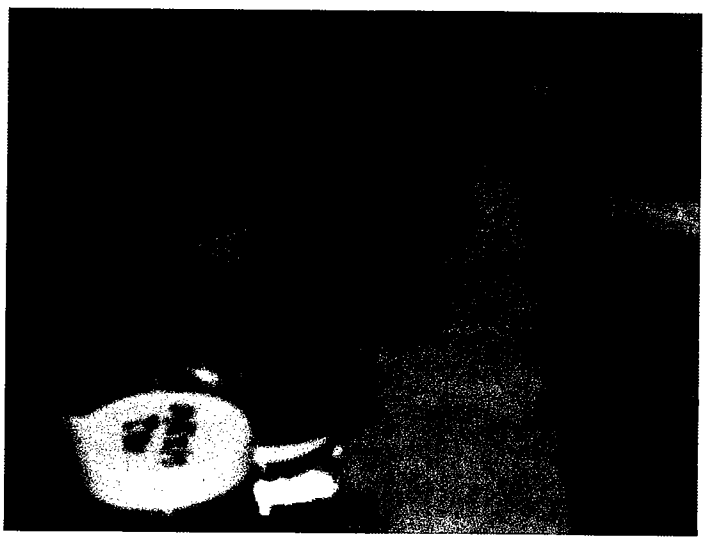


Figure 3.10. Example of a Ferrate Treatment Sample After Shaking. Orange color indicates that the ferrate reagent has reacted (compare to Figure 3.9).

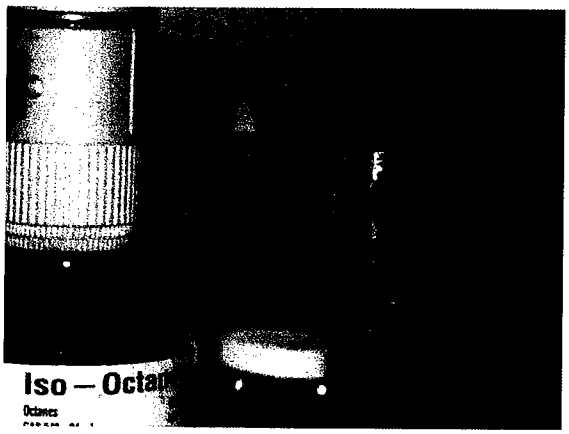


Figure 3.11. Addition of Isooctane to a Ferrate Treatment Sample HD for preparation of sample for GC analysis.

3.4.6 Objective of Decontamination Product Analysis by Full Scan (FS) GC-MS

FS GC-MS testing analyses of decontamination samples were conducted to:

- Quantitatively determine the amount of HD in the organic phase to determine the percent destruction of HD
- Qualitatively determine the degradation products of HD in the organic phase, such as divinyl sulfone (DVSO2), thiodiglycol (TDG), thiodiglycol sulfone (TDGO2), dithiane, and thioxane. Past Battelle experience has shown that many of the potential 2-chloro and hydroxy-intermediates that could occur during the S-oxidation steps of HD are known to be unstable and rapidly hydrolyze or dehydrohalogenate to 2-hydroxy and vinyl compounds. These compounds were not observed as was expected. This is the reason that standards for them were not found even though a fresh search was made. For project thoroughness, these compounds were searched for in the full scan GC-MS.

Table 3.3 summarizes the FS GC-MS work conducted at the HMRC. Figure 3.12 illustrates a standard total ion chromatogram analysis (TICA) of HD and HD degradation products. In addition, a detailed sample analysis flow scheme for HD is provided in Appendix B.

Table 3.3. Summary of HMRC Decontamination Analysis by FS GC-MS

Sample Phase	Agent	Instrument/Mode	Analysis
Iso-Octane Extract	HD	GC-MS/Full Scan	Quantitatively to determine % destruction of agent
Iso-Octane Extract	HD	GC-MS/Full Scan	Qualitatively to determine agent and pertinent readily identified organics

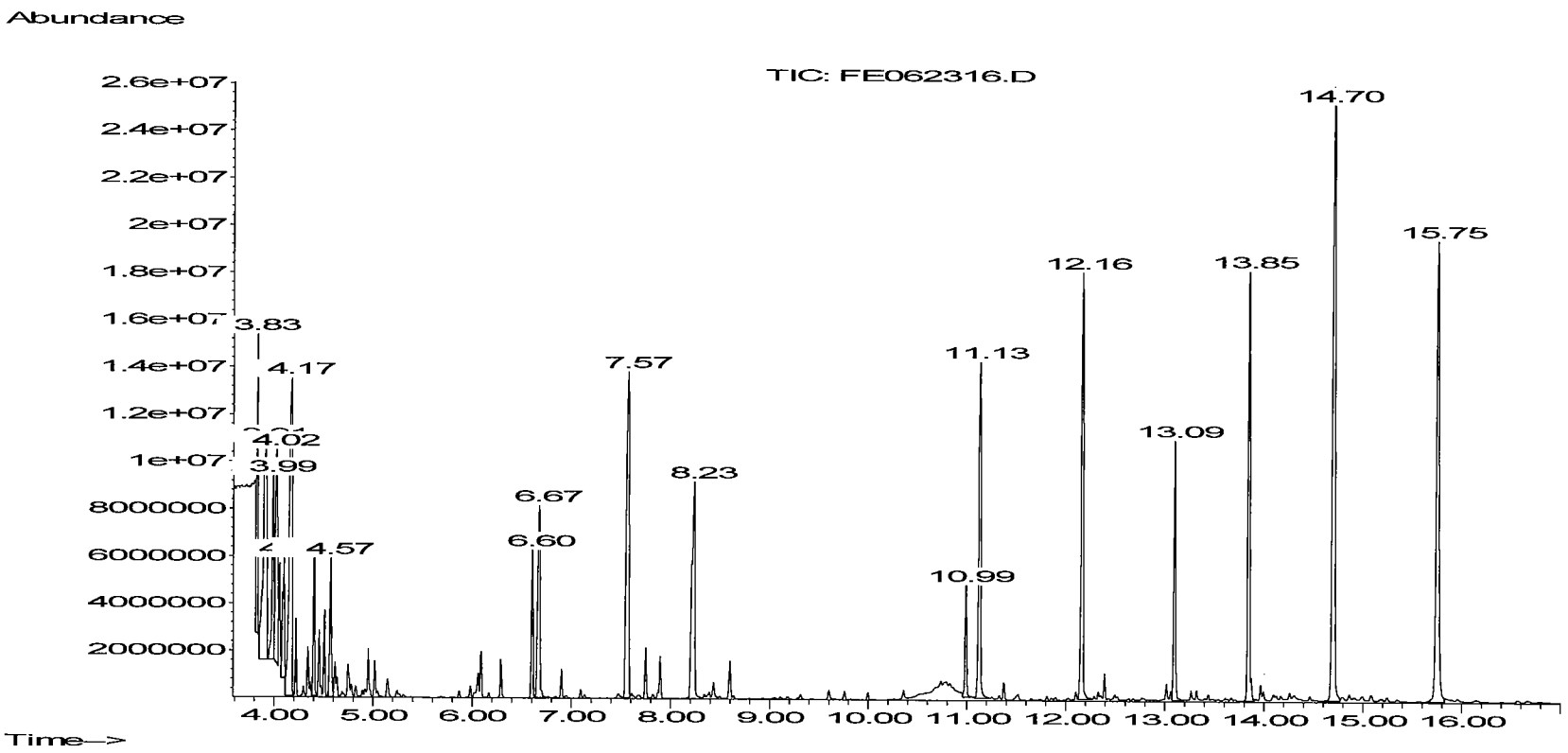


Figure 3.12. Standard TICA of HD and HD Degradation Compounds by FS GC-MS. Y-axis is ion abundance and X-axis is GC retention time.

6.60 Octane, 1-chloro
6.67 1-Octanol
7.56 Mustard Gas
8.23 1-Undecanol
10.99 Sesquimustard
11.13 1-Octanamine, N-methyl-N-octyl
12.16 No match
13.09 No match
13.85 !-Octanamine, N,N-dioctyl
14.70 No match
15.75 No match

3.4.7 FS GC-MS Quality Assurance/Quality Control (QA/QC)

Following completion of the decontamination testing, the GC vials with CoC forms were submitted to the analyst responsible for maintaining possession of the samples. The analyst reviewed the CoC form against the identifiers on the GC vials to ensure matching lists. Finally, the organic phase of both the HD and VX was analyzed by GC-MS.

The GC-MS was calibrated prior to daily analysis. Five calibration standards were run for HD at 1.0, 2.5, 5.0, 10.0, and 25 µg/mL, and a linear regression forced through zero was used to quantify the data. Check standards were run after every five samples. The purpose of the spike controls was to verify that the correct amount of agent was dispensed by the manual dispenser. For VX, four calibration standards were run at levels of 1.0, 2.5, 5.0, and 10.0 µg/mL. A quadratic fit was used. All analytical standard solutions were placed in individual automatic liquid sampler (ALS) vials, stored in a freezer at -22°C, and only removed just prior to use. Standard solutions removed from the freezer were used within 48 hr and then decontaminated.

Following completion of the decontamination analysis by FS GC-MS, the analyst updated the CoC forms and LRB by recording the date of analysis, data management file number in which the analytical data were recorded, instrument number, and calibration date. In addition, select samples were sent to BCO analytical laboratories for further analysis of reaction products by liquid-chromatographic electrospray-ionization mass-spectrometry (LC-ESI-MS-MS).

3.4.8 GC-MS Test Parameters

Table 3.4 delineates the GC-MS parameters used for decontamination testing at the HMRC.

Table 3.4. HMRC Decontamination Testing GC-MS Parameters

GC-MS Conditions	
GC-MS Model	Agilent 5973N
Column	ZB-5 column, 30 meters x 0.25 mm ID, 25 μm film
Carrier Gas	Helium
Injection Temperature	250°C
Injection Volume	1μL
Injection Mode	Splitless
	40°C Hold at 40°C for 2 min 40°C to 280°C at 20°C/min Hold at 280°C for 3 min
Retention Time	HD: 7.55 min and VX: 10.91 min
Transfer Line Temperature	280°C
EI	70eV
Source Temperature	230°C
Quadrpole Temperature	150°C
Solvent Delay	3.5 min.
Electron Multiplier	EM voltage at 200 above autotune
Acquisition Mode	Full Scan
Scan Range	45 to 525 Daltons

3.5 Decontamination Product Analysis by LC-MS-MS

3.5.1 Objective

Decontamination product analysis by LC-ESI MS-MS was carried out at the BCO. LC-ESI MS-MS testing analyses of HD decontamination samples were conducted quantify degradation products (divinyl sulfone or DVSO2; Thiodiglycol or TDG; and Thiodiglycol sulfone or TDGO2) in the aqueous phase of samples (Table 3.5). Figures 3.13 and 3.14 display a standard TICA and multiple reaction monitoring (MRM) chromatogram of HD degradation products, respectively. The LC-MS-MS analyses of decontaminated VX samples were conducted to identify quantitatively select degradation products (ethoxy methylphosphonic acid or EMPA, 2-N,N-diisopropylaminoethanol or DIPAE, and VX acid or EA 2192) in addition to VX (Table 3.6). Figures 3.15 and 3.16 display a standard TICA and MRM chromatogram of VX and VX degradation products, respectively. The HD and VX

methods followed for residue analysis can be found in Appendices C and D, respectively.

Table 3.5. HD Degradation Compounds Analyzed by LC-MS-MS

Compound	Ionization Method	Method Detection Limit	M+1 Parent Mass	Comments
Divinyl Sulfone	APCI*	25 ppb	119	
Thiodiglycol	APCI	25 ppb	123	
Bis(2-hydroxy ethyl) Sulfone	APCI	100 ppb	155	Ionization by APCI only

*APCI: Atmospheric Pressure Chemical Ionization

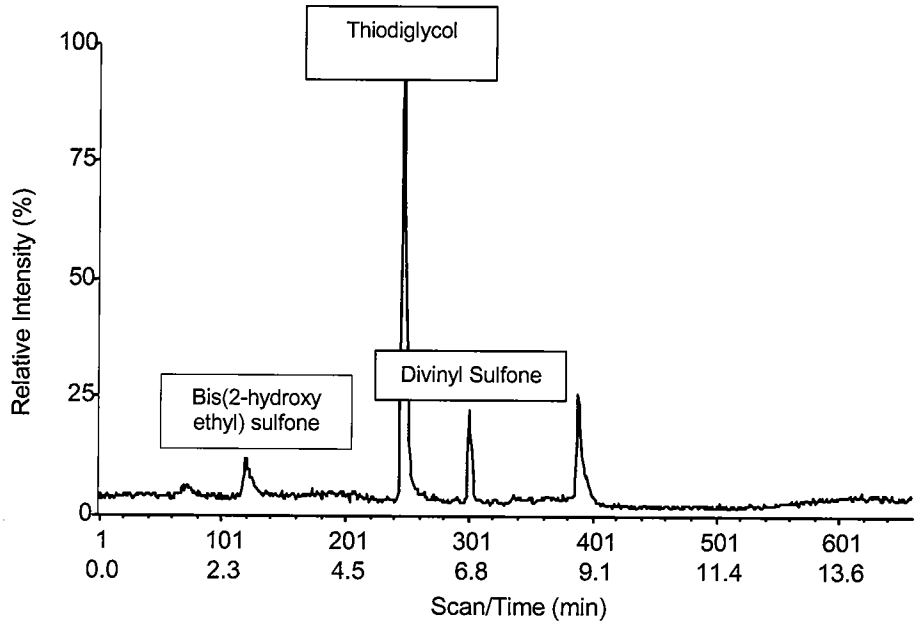


Figure 3.13. Standard TICA of HD Degradation Compounds by LC-MS-MS.

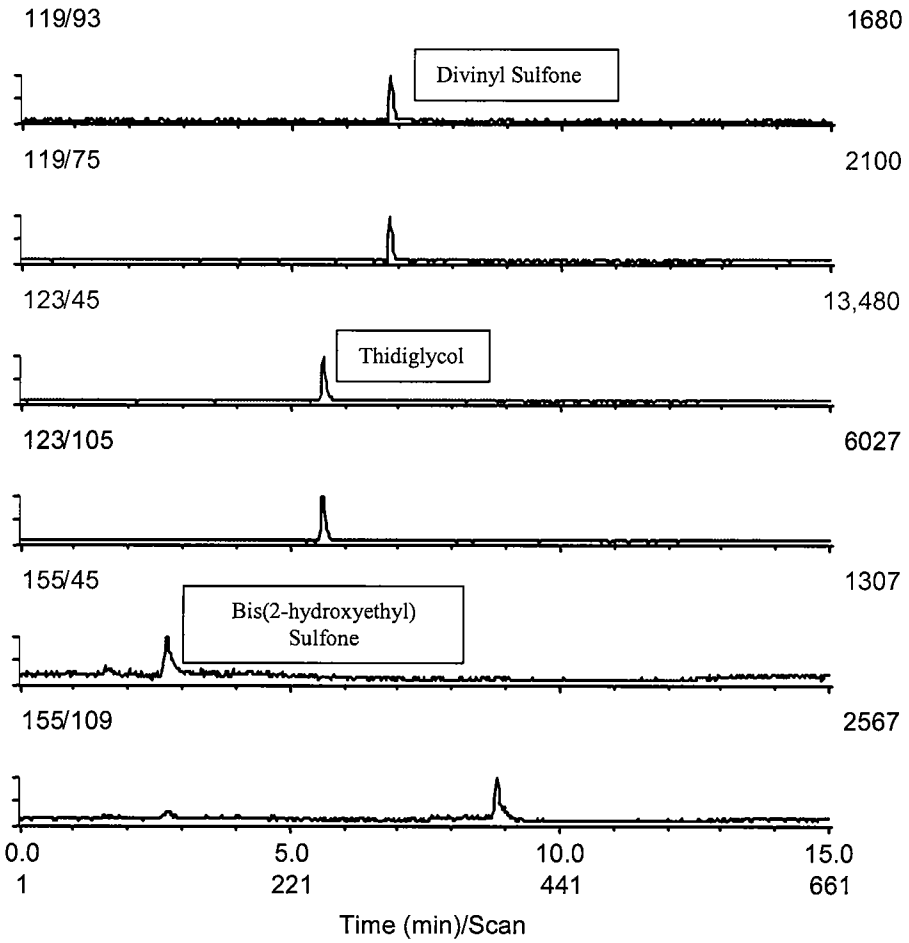


Figure 3.14. Standard MRM Chromatograms of HD Degradation Compounds by LC-MS-MS.

Table 3.6. VX Degradation Compounds Analyzed by LC-MS-MS

Compound	Ionization Method	Method Detection Limit	M+1 Parent Mass	Comments
EA-2192	ESI	1 ppb	240	Ionization by ESI only
Diisopropyl amino ethanol	ESI	1 ppb	146	—
VX	ESI	1 ppb	268	—
Ethyl methylphosphonate	ESI	5 ppb	125	—

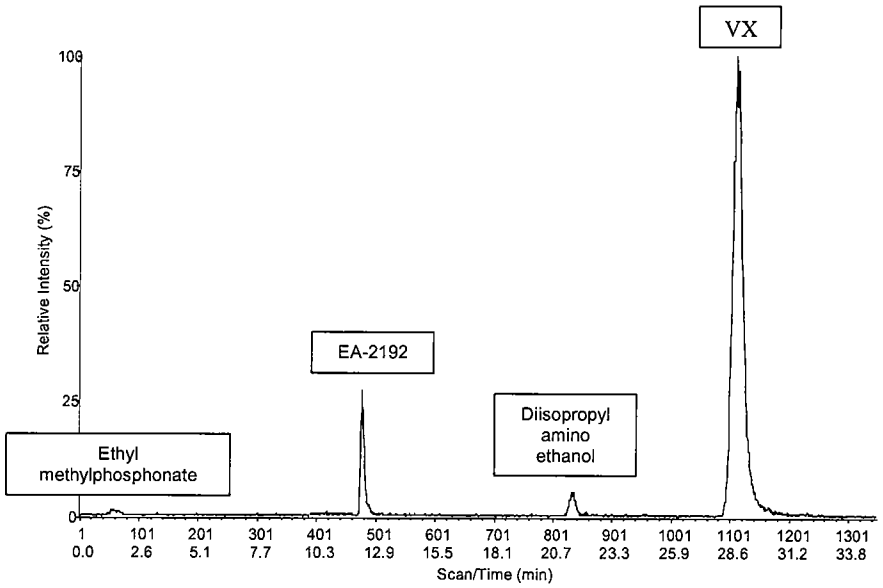


Figure 3.15. Standard TICA of VX and VX Degradation Compounds by LC-MS-MS

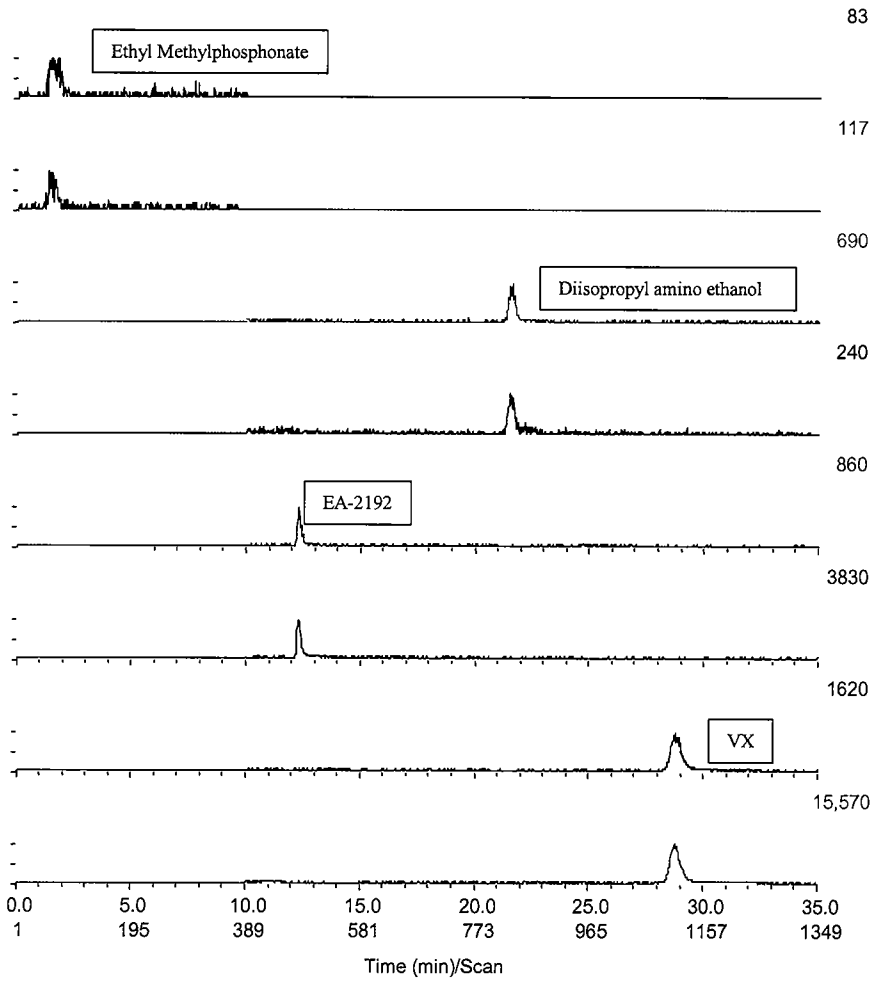


Figure 3.16. Standard MRM Chromatograms of VX Degradation Compounds by LC-MS-MS.

3.5.2 Sample Transfer and Storage

Samples were transferred from the HMRC to BCO according to ECBC requirements. A CoC stating the sample identifier, agent type, and agent estimated test concentration accompanied the samples. All samples were stored at -20°C (± 3°C) in Freezer 44 (monitored), Laboratory 20-2-44. Samples were allowed to equilibrate to room temperature prior to analysis.

3.5.3 Test Equipment

The residue analysis was performed using SCIEX API III+ Triple Quadrapole Mass Spectrometers (Figure 3.17). VX and the products of the HD and VX analysis utilized different ionization techniques: APCI for the HD products and ESI for VX and VX products. The differing techniques were employed because some compounds responded more favorably by one type of ionization than another. Samples were introduced to MS via Shimadzu LC-10AD LC, adding another dimension of selectivity to the analysis.

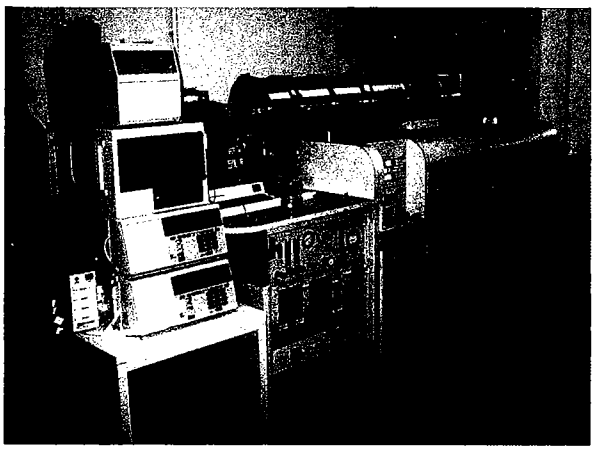


Figure 3.17. SCIEX API III+ LC-MS-MS System.

3.5.4 LC-MS-MS QA/QC

Prior to each analysis by LC-MS-MS, the mass assignment of the instrument was verified by ionizing compounds of known masses. Samples and standards were removed from the freezer and brought to room temperature before transfer to

autosampler vials or dilution. All standard preparation and sample dilution were performed using calibrated pipettes and/or class A volumetric glassware. Each analysis sequence began with a high-purity, deionized (DI) water blank and appropriate standards to ensure instrument performance. Bracketing standards were used throughout each sequence to monitor sensitivity changes and for quantitation. In the event that the analysis sequence failed to meet quality criteria, the affected samples were re-analyzed.

4 RESULTS AND DISCUSSION

The project results are organized according to project deliverables. The thermal shelf life results are provided first followed by the CWA decontamination results.

4.1 K_2FeO_4 Thermal Stability Testing According to AR 70-38

To determine hot storage shelf-life for ferrate, two long-term hot thermal stability tests, isothermal and temperature cycling, were performed in triplicate. These measurements were performed on samples of K_2FeO_4 (Technical Grade, TG) consistent with AR 70-38 Sec. II, Table 2-2 (Storage and Transit Conditions). It was not necessary to use high purity product.

The purity of K_2FeO_4 TG over time at the isothermal (71°C) testing condition is shown in Table 4.1 and Figure 4.1:

Table 4.1. Isothermal Test Results for Ferrate Decontamination Reagent Active Component (K_2FeO_4) at Various Initial Purities Indicative of "Technical Grade" (measured in triplicate).

Elapsed Time at 71°C (days)	Vial 1 Average Purity (%)	Vial 1 Std Dev (%)	Vial 2 Average Purity (%)	Vial 2 Std Dev (%)	Vial 3 Average Purity (%)	Vial 3 Std Dev (%)	Vial 4 Average Purity (%)	Vial 4 Std Dev (%)
0	92.60	0.59	87.55	1.01	90.24	1.58	74.57	2.82
1	93.99	0.60	91.04	0.52	89.59	2.69	77.57	1.35
2	95.15	1.02	91.13	1.48	94.07	1.51	79.01	0.94
5	90.66	3.40	89.71	0.75	89.40	1.00	71.84	0.58
7	90.94	1.98	90.98	0.10	92.41	1.83	76.96	0.96
12	91.84	0.65	93.06	0.10	92.53	2.31	76.41	1.17
27	88.56	0.88	88.59	0.08	89.69	1.49	76.36	2.78
48	87.07	0.78	86.75	2.59	84.73	2.13	74.92	2.55
98	83.44	2.36	86.99	1.76	80.57	2.49	73.64	0.53

The K_2FeO_4 remaining after 98 days of storage at 71°C was 94.4%. Degradation of ferrate crystals under isothermal 71°C storage after 98 days ranged from 0% for the material having an initial purity of 79.6% to 10.7% for the initially 90.24% pure sample. After 98 days at 71°C, the average decrease in purity among the four samples was 5.6±5.4% indicating good storage stability for ferrate crystals across a range of initial purities ranging from 74% to 93%.

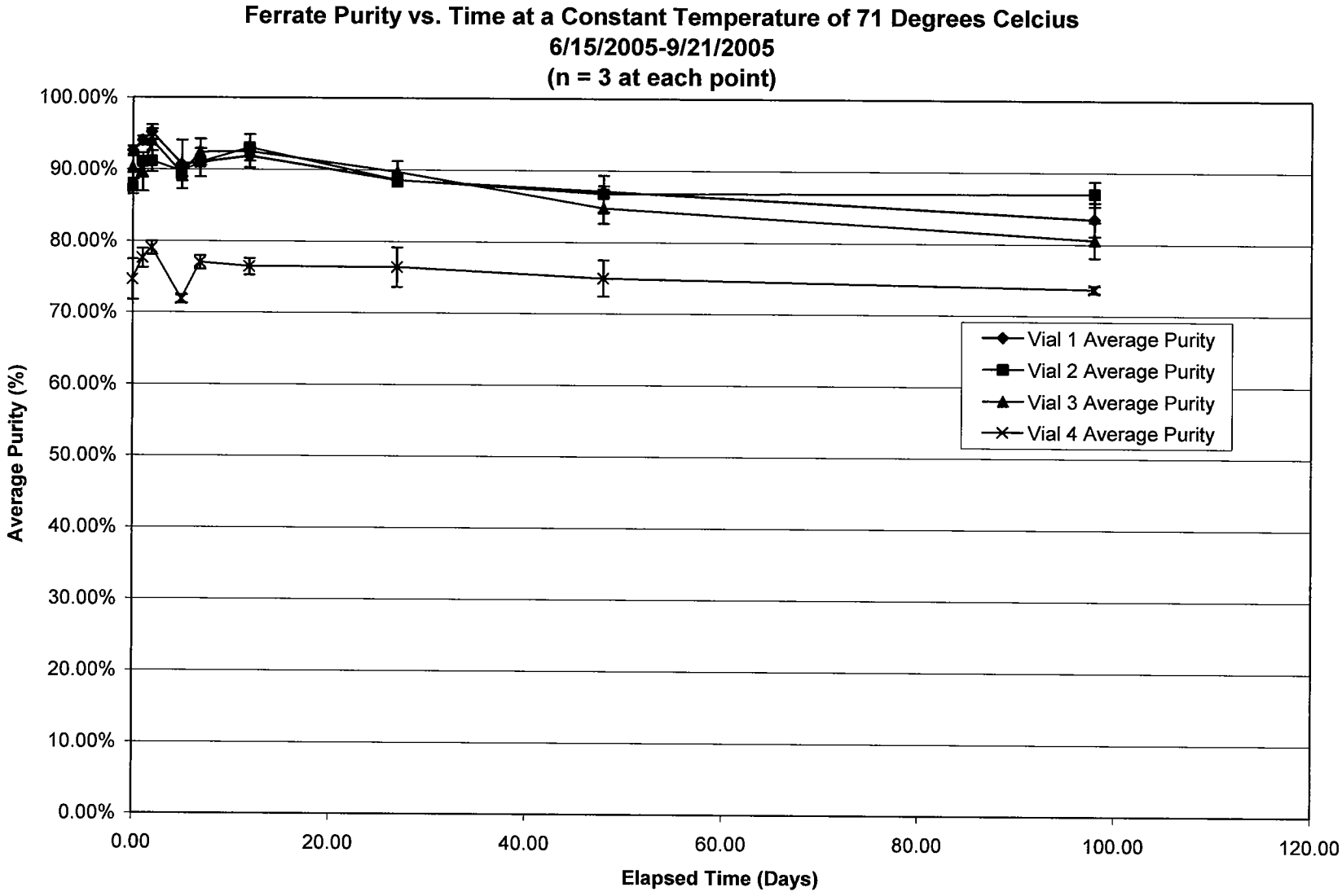


Figure 4.1. Thermal Stability Test Results for Technical Grade Potassium Ferrate

The data definitively indicate that K_2FeO_4 TG is stable under the hottest environmental conditions (71°C) specified by AR 70-38. It is significant that this stability is exhibited by material fairly pure and also not nearly pure. Normally reactive materials, for example peroxides, tend to decompose rapidly when impure. The ability to use TG material will help keep cost of implementation low, as the product will not need to be highly purified or packaged using very high purity materials and techniques. This stability is tentatively attributed to the product being a crystalline material and the ferrate ion, $FeO_4^{=}$, being of high symmetry (tetrahedral, Td).

The data for the second temperature stability study involving cycling temperature appears in Table 4.2 and Figure 4.2. After 82 days of storage under cyclic temperature conditions, the potassium ferrate retained an average 93.1% of its initial purity.

Table 4.2. Temperature-Cycling Testing Summary (n = 3)

Elapsed Time (Days)	Overall Average Purity (%)	Std Dev [b] (%)	RSD[c] (%)
0	92.7	0.8	0.8
11	94.7	4.2	4.4
32	89.1	0.9	1.0
82	86.3	1.4	1.7

[a] Testing stopped due to termination of project.
[b] Std Dev = Standard deviation
[c] RSD = Relative Standard Deviation

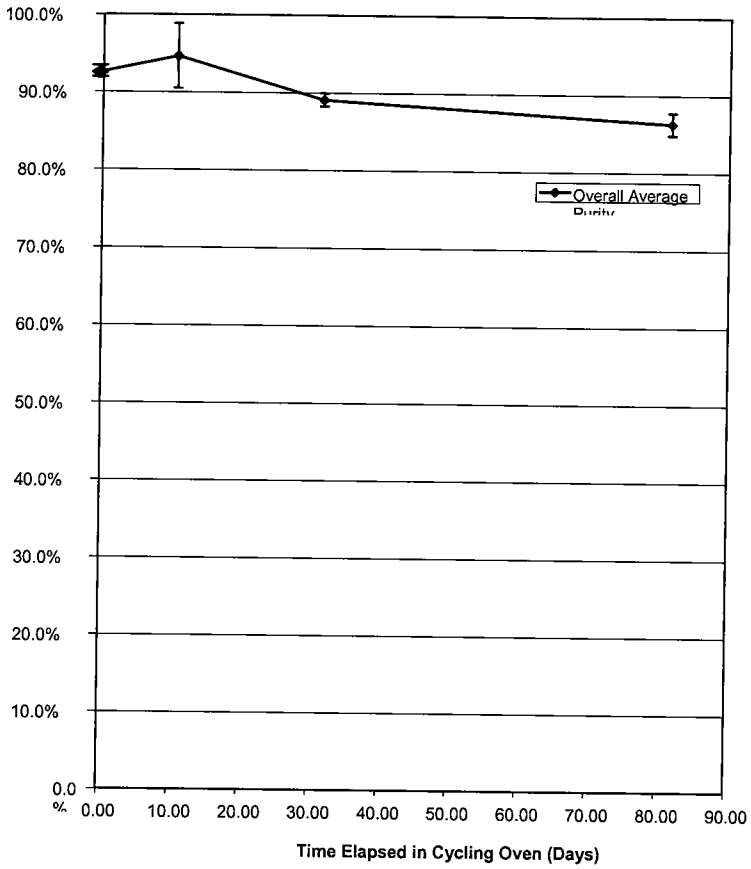


Figure 4.2. Thermal Stability Test Results for Potassium Ferrate Decontamination Reagent at AR 70-38 Temperature Cycling (71°C maximum temperature each day) for 82 Days (n=3)

After being stored 82 days under the controlled cyclic temperature condition, $\frac{86.3}{92.7} x100\%$ of the original ferrate remained intact. As with the isothermal test results, the data suggest that K_2FeO_4 TG crystals are stable under the thermal conditions of AR 70-38 Sec. II, Table 2-2 (Storage and Transit Conditions).

4.2 Decontamination Testing of HD Using a Ferrate Formulation with Product Analysis by FS GC-MS and LC-MS-MS

4.2.1 Run 1: HD with Final pH Moderately Alkaline

4.2.1.1 Decontamination Testing with Analysis by GC-MS

Per the procedures described in Section 3.2.2 and 3.4.5, 3.85 mg/mL of HD and a 22.5:1 mass ratio of ferrate to HD was used. As expected, all three ferrate treatments (samples FT-1, FT-2, and FT-3) turned a deep purple color upon ferrate addition. Also as expected, a small amount of gas release was heard when the vial cap was loosened after reaction due to a small amount of oxygen gas generation. (Caused by the low initial pH (<7) of the buffered system. Based on the results below, the buffer may be omitted in future formulation refinements since problematic by-products were not found, making such gas formation a non-issue). Within 10 minutes of shaking, the color had turned brown, indicating the ferrate had reacted and reduced the ferric hydroxide. The non-ferrate buffer references (samples pH-1, pH-2, and pH-3) became cloudy white after the final vortexing step and remained such through the 60-min shaking period. The water references (samples WR-1, WR-2, and WR-3) remained clear through all steps, with HD beading up in the water, as expected due to its low water solubility. The negative control (samples NC-4, NC-5, and NC-6) observations were identical to the ferrate treatments but with no agent added.

The ferrate treatment (with an initial pH of < 10.5 and a final reaction pH of 12.4±0.1) (Table 4.3), resulted in an HD decontamination level of 99.1±0.2%. In the case of the non-ferrate buffer reference [with initial pH of 10.5 and final reaction pH of 9.9±0.2], the HD decontamination level of 88.9 ± 0.5% was lower than the ferrate-treatment by 10% absolute. Even without the addition of buffer or ferrate, i.e. water-only reference (final pH of 2.1±0.2), a significant amount of HD removal occurred, 88±15%, but with an indication of more variability within the three replicates (96.51%, 97.08%, and 70.63%) (Table 4.3). Supporting FS GC-MS qualitative product analysis results (Table 4.4) indicated the absence of degradation products of concern, DVSO2, dithiane, and thioxane, for the ferrate treatments.

Water is notorious for slow reaction rates and lack of consistency in decontaminating HD because of skinning over of the dispersed HD droplets by polymerization reactions in the water in which HD is poorly soluble, yet reactive. Therefore, as expected, the pH of the water reference samples plummeted to 2.1±0.2, due to the formation of HCl, a highly corrosive material. We note that the ferrate-generated base neutralizes any acids that form, such as HCl.

Table 4.3. HD Decontamination Test Results by GC-MS Using Ferrate

Sample Description	Sample ID	Run	pH After Reaction	HD Added (mg)	HD Remaining (mg)	HD Removed (%)	K_2FeO_4 Decontamination: Agent Ratio
Ferrate Trt*	FT-1	1	**12.3**	11.54	0.13	98.87	22.5
Ferrate Trt	FT-2	1	**12.5**	11.54	0.10	99.10	22.5
Ferrate Trt	FT-3	1	**12.4**	11.54	0.09	99.19	22.5
				Average→	**0.11**	**99.1**	
				Std Dev→	**0.02**	**0.2**	
pH 10.5 Ref.	pH-1	1	**9.9**	11.54	1.24	89.26	0
pH 10.5 Ref.	pH-2	1	**9.9**	11.54	1.35	88.30	0
pH 10.5 Ref.	pH-3	1	**9.9**	11.54	1.25	89.17	0
				Average→	**1.28**	**88.9**	
				Std Dev→	**0.1**	**0.5**	
Water Ref.	WR-1	1	**2.3**	11.54	0.40	96.51	0
Water Ref.	WR-2	1	**2.0**	11.54	0.34	97.08	0
Water Ref.	WR-3	1	**2.1**	11.54	3.39	70.63	0
				Average→	**1.9**	**88.1**	
				Std Dev→	**1.7**	**15.1**	
Negative Control	NC-1	1	**13.1**	0.00	ND	NA	0
Negative Control	NC-2	1	**13.1**	0.00	ND	NA	0
Negative Control	NC-3	1	**13.1**	0.00	ND	NA	0
Ferrate Trt.	FT-4	2	**7.0**	11.54	2.33	79.78	22.5
Ferrate Trt.	FT-5	2	**7.0**	11.54	2.44	78.83	22.5
Ferrate Trt.	FT-6	2	**7.0**	11.54	2.28	80.21	22.5
				Average→	**2.4**	**79.6**	
				Std Dev→	**0.1**	**0.7**	
pH 7 Ref.	pH-4	2	**7.8**	11.54	6.56	43.17	0
pH 7 Ref.	pH-5	2	**8.4**	11.54	6.33	45.16	0
pH 7 Ref.	pH-6	2	**8.4**	11.54	6.56	43.17	0
				Average→	**6.5**	**43.8**	
				Std Dev→	**0.1**	**1.2**	
Ferrate Trt.	FT-7	3	**7.0**	98.11	39.5	59.74	2.7
Ferrate Trt.	FT-8	3	**7.0**	98.11	45.1	54.03	2.7
Ferrate Trt.	FT-9	3	**7.0**	98.11	37.2	62.08	2.7
				Average→	**40.6**	**58.6**	
				Std Dev→	**4.1**	**4.1**	
pH 10.5 Ref.	pH-7	3	**6.9**	98.11	79.3	19.17	0
pH 10.5 Ref.	pH-8	3	**6.7**	98.11	63.5	35.28	0
pH 10.5 Ref.	pH-9	3	**7.0**	98.11	64.8	33.95	0
				Average→	**69.2**	**29.5**	
				Std Dev→	**8.8**	**8.9**	

ND: Not-detect
NA: Not Applicable
Std Dev: Standard Deviation

*Ferrate Trt: Ferrate Formulation Treatment as per test protocol provided in Section 3.2.2 of this report.

Table 4.4. HD and VX Qualitative Decontamination Product Results Using Ferrate and Reference Systems and Qualitatively[6] Identified by FS GC-MS[1] of Iso-Octane Extracts[2]

Assign-ment	Test Sample Identification ⇨	HD Run 1				HD Run 2		HD Run 3		VX Run 4			
		Ferrate Treatment	pH 10.5 Reference	Water Reference	Negative Control	Ferrate Treatment	pH 7 Reference	Ferrate Treatment	pH 10.5 Reference	Ferrate Treatment	pH 7 Reference	Water Reference	Negative Control
Probable Source of Com-pound	Replicate Identification ⇨	FT-1, FT-2, and FT-3	pH-1, pH-2, and pH-3	WR-1, WR-2, and WR-3	NC-1, NC-2, and NC-3	FT-4, FT-5, and FT-6	pH-4, pH-5, and pH-6	FT-7, FT-8, and FT-9	pH-7, pH-8, and pH-9	FT-10, FT-11, and FT-12	pH-10, pH-11, and pH-12	WR-4, WR-5, and WR-6	NC-4, NC-5, and NC-6
HD	1,4-Dithiane	ND	ND	ND	ND	ND	X	ND	ND	NA	NA	NA	NA
PTC	1-Octanamine, N,N-dioctyl-	X	X	X	X	X			X	X			X
PTC	1-Octanamine, N-methyl-N-octyl-	X	X	X	X	X		X	X	X			X
TBD	1-Undecanol							X		X			X
TBD	2-Dodecene, (Z)-									X			X
Extraction Solvent	Cyclooctane								X	X			X
TBD	Cyclopropane,1-methyl-2-octyl									X			
TBD	Decane, 1-chloro					X				X			X
TBD	Decanoic acid, decyl ester									X			X
HD	Divinyl Sulfone (DVSO2)	ND	ND	ND	ND	ND	ND	X	ND	NA	NA	NA	NA
HD	HD	X	X	X	ND	X	X	X	X	NA	NA	NA	NA
Extraction Solvent	Octanoic acid, octyl ester									X			
VX	O-Ethyl S-2-diisopropylaminoethyl ethylphosphonothiolate	NA	NA	NA	NA	NA	NA	NA	NA	ND	X	X	ND
HD	Sesquimustard	ND	ND	ND	ND	X	X	X	ND	NA	NA	NA	NA
HD	Thiodiglycol (TDG)	ND	ND	ND	ND	ND	ND	ND	ND	NA	NA	NA	NA
HD	Thioxane	ND	ND	ND	ND	ND	ND	ND	ND	NA	NA	NA	NA
VX	VX [3]	NA	NA	NA	NA	NA	NA	NA	NA	ND	ND	X	ND

[1] These results include both significant and insignificant, but detectable, amounts of the compounds found. (Quantitative analyses are provided in the following tables for key and major compounds.)

[2] "X" corresponds to present (Tentative identification based on NIST library reverse search with match criteria of generally 80% or higher)
Many of the compounds listed are believed to be associated with the iso-octane extraction solvent. Not all solvent-related compounds identified were included in this table. Future testing should use a less complex extraction solvent.
The P-ethyl impurity in the stock VX was also destroyed by ferrate as it did not show up in this GC chromatogram..
Detection limit is 0.5 µg/ml.

[3]Note that VX results are very qualitative since VX does not GC well due to the high water solubility of the VXH^+ cation formed at medium pH values. Therefore LC-based assays were used for VX (see below).

4.2.1.2 HD Decontamination Products for Run #1: Quantitative Product Analyses by LC-MS-MS

According to the LC-MS-MS results for ferrate-treatment (samples FT-1, FT-2, and FT-3) in Table 4.5, an absence was noted for the desirable products, divinyl sulfone (DVSO2) (<13 µg/mL), thiodiglycol (TDG) (<13 µg/mL), and thiodiglycol sulfone (TDGO2). However, consistent with literature predictions, problematic toxic TDG was formed at similar concentrations in both the non-ferrate buffer treatment and the water-reference treatment samples (pH-1, pH-2, and pH-3 averaging 910 ± 105 µg/mL, and WR-1, WR-2, and WR-3 averaging 1160 ± 460 µg/mL respectively). In addition, Table 4.5 revealed that TDGO2 and DVSO2 were absent for both the non-ferrate buffer reference (<133 µg/mL and <33 µg/mL, respectively) and the water-reference (<667 µg/mL and <167 µg/mL, respectively). As required, negative control samples (NC-1, NC-2, and NC-3) were absent for TDG (<0.25 µg/mL). Likewise, DVSO2 (<0.25 µg/mL) and TDGO2 (<0.25 µg/mL) were absent in all of the negative control samples. Hence, since the full scan GC and LC did not show the presence of additional organic products, beyond not producing toxic products, ferrate largely converted HD nontoxic small molecules and/or full mineralization.

The amount of buffer employed in Run 1 was found to be insufficient to prevent the pH from rising to 12.4 ± 0.1, above the objective pH of 10.5. We note however that pH 9-10 is an unbuffered region for the chemistry under consideration. Since toxic products were not found in Run 1, the need to control pH may not be critical to controlling toxic products formation when ferrate is the decontamination reagent. Also, Run 1 demonstrated the capacity for ferrate to generate hydroxide ions, useful for hydrolysis agent decontamination activity in addition to oxidation, and in neutralizing acids so formed. This illustrates how ferrate thereby provides several decontamination chemistries that boost its decontamination activity per unit weight of reagent.

Even at a final pH of 12.4, toxic products from HD were not found in ferrate treated samples. This dual capacity to provide hydroxide ion for hydrolysis and acid neutralization reactions and oxidant for fast decontamination reactions highlights the

potential for a high level of agent decontamination per unit weight of ferrate. For HD, water and high pH readily form a large yield of toxic products. However, toxic products are not formed at high pH when ferrate is present.

Important differences were noted about the final products when comparing results of HD with and without ferrate. Only extractant solvent organic products could be found by FS GC-MS (Table 4.4), and no target analytes by LC-MS-MS for samples with ferrate (FT-1, FT-2, FT-3, NC-1, NC-2, and NC-3) (Table 4.5). However, for samples with no ferrate (encompassing non-ferrate buffer [samples pH-1, pH-2, and pH-3] and water reference [samples WR-1, WR-2, and WR-3]) toxic or potentially toxic products were formed. For the non-ferrate buffer, TDG (an undesirable product that can revert to HD or polymerize to form toxic compounds) averaged 910 ± 105 µg/mL, with a yield of 103 ± 12%, and produced no desirable DVSO2 or TDGO2.

4.2.2 Run 2: HD Decontamination Using Ferrate with Final pH of 7

4.2.2.1 Decontamination Testing by FS GC-MS.

For Run 2, 3.85 mg/mL (0.024M) of HD and a 22.5:1 mass ratio of ferrate to HD were used. Observations for all three ferrate replicates (samples FT-4, FT-5, and FT-6) were identical to those of Run 1, except that bubbling was observed when the ferrate was added. This gassing was caused by the higher amount of acid phosphate buffer added to attain a final pH closer to neutrality. The observations for the non-ferrate buffer reference (samples pH-4, pH-5, and pH-6) were identical to those of Run 1 despite the lower final pH of Run 2 indicating that the pH or the phosphate ion of the buffer did not measurably affect the reaction path, the reaction being dominated by hydrolysis in this pH range and at ambient temperature.

The ferrate treatment of Run 2, with final reaction pH = 7.0 ± 0.1 (Table 4.3), resulted in a significant but lower HD decontamination level of 80 ± 1% than the 99.1% found for Run 1. In the case of the non-ferrate buffer reference [pH 8.2 ± 0.2], HD decontamination resulted in a substantial drop to 44 ± 1% decontamination, or about ½ the decontamination attained by the ferrate treatment at otherwise similar conditions. Supporting FS GC-MS qualitative results indicate the absence of known

HD degradation products, i.e. DVSO2, dithiane, and thioxane with the exception of detecting dithiane in the non-ferrate buffer reference samples (Table 4.4). Hence, as with Run 1 conditions, Run 2 conditions also did not result in problematic toxicity products when ferrate was used, and the yield of decontamination with ferrate is far better than water hydrolysis at neutral pH.

4.2.2.2 Run 2: Decontamination of HD with a Final pH 7. Quantitative Product Analyses by LC-MS-MS.

The LC-MS-MS results in Table 4.5 revealed that the less desirable TDG was detected at an average concentration of 196 ± 70 µg/mL at the lower pH of Run 2, giving an average yield of 22 ± 8% in the ferrate treatment (samples FT-1, FT-2, and FT-3) based on the starting amount of HD introduced.

> **NOTE:** Yield in this case is relative to the case where if 100% of the agent is converted to the product for which the percentage is given. High percentages are desired for low toxicity compounds and low to zero percentages are desired for high toxicity compounds.

In addition, Table 4.5 revealed that DVSO2 and TDGO2 were absent (<53 µg/mL and <13 µg/mL, respectively) in the ferrate treatment. However, undesirable TDG was formed 17 times greater in the non-ferrate buffer treatment, (samples pH-5 and pH-6) (1,010 ± 54 µg/mL) than in the ferrate-treatment. This amount represents ~100% (114 ± 6%) of HD to TDG in the case of non-ferrate reference. Similar to the ferrate treatment samples, DVSO2 and TDGO2 were absent (<167 µg/mL and <667 µg/mL, respectively) in the non-ferrate buffer samples (Table 4.5).

Hence, the results reveal that the TDG levels for non-ferrate buffer samples are 17 times greater than the TDG levels for ferrate treated samples at the test conditions of pH7.

Since significant gassing was observed, and this is known to be due to the very low starting pH (~3) provided by the orthophosphate, monobasic pH buffer used in an attempt to provide a final pH of the reaction mixture of about 7 vs an alkaline pH as was the objective in Run 1. Since Run 2 results were lower yielding than Run 1 results, and incompletely reacted HD and HD intermediates were found in Run 2 but not Run 1, this result suggests that the lower pH did not enhance the desired

decontamination chemistry despite the use of the same amount of ferrate reagent. In fact, the gassing may indicate a significant loss of ferrate material by the water oxidation to O_2 side reaction due to the low pH effect on increasing ferrate's oxidation potential. As a result, insufficient ferrate remained to achieve the full HD decontamination. In future formulation work for the ferrate reagent (out of scope for this project), the acidic buffer should be omitted or at least reduced. Less acid buffer and less ferrate decomposed in a side reaction provides a very active reagent, as evidenced by the Run 1 results. This change would increase the decontamination capacity of ferrate ion as well without the risk of generating toxic degradation products at the higher pH. Alternatively, more ferrate could be used at the lower pH.

Table 4.5. HD Decontamination Product Results by LC-MS-MS Using Ferrate Decontamination Reagent

Sample Description	Sample ID	Run #	Initial HD Added (µg/mL)	Maximum DVSO2 possible (µg/mL)	FOUND DVSO2 by LC-MS-MS (µg/mL)	Yield of DVSO2 (%)	Maximum TDG possible (µg/mL)	FOUND TDG by LC-MS-MS (µg/mL)	Yield of TDG (%)	Maximum TDGO2 possible (µg/mL)	FOUND TDGO2 by LC-MS-MS (µg/mL)	Yield of TDGO2 (%)
Ferrate Trt.	FT-1	1	3847	856.9	ND	NA	886.1	ND	NA	1118.3	ND	NA
Ferrate Trt.	FT-2	1	3847	856.9	ND	NA	886.1	ND	NA	1118.3	ND	NA
Ferrate Trt.	FT-3	1	3847	856.9	ND	NA	886.1	ND	NA	1118.3	ND	NA
				Average	NA	NA		NA	NA		NA	NA
				Std dev	NA	NA		NA	NA		NA	NA
pH 10.5 Ref.	pH-1	1	3847	856.9	ND	NA	886.1	892	100.7	1118.3	ND	NA
pH 10.5 Ref.	pH-2	1	3847	856.9	ND	NA	886.1	816	92.1	1118.3	ND	NA
pH 10.5 Ref.	pH-3	1	3847	856.9	ND	NA	886.1	1023[1]	115.4	1118.3	ND	NA
				Average	NA	NA		910	102.7		NA	NA
				Std dev	NA	NA		105	11.8		NA	NA
Water Ref.	WR-1	1	3847	856.9	ND	NA	886.1	1043	117.7	1118.3	ND	NA
Water Ref.	WR-2	1	3847	856.9	ND	NA	886.1	1663[1]	187.7	1118.3	ND	NA
Water Ref.	WR-3	1	3847	856.9	ND	NA	886.1	771	87.0	1118.3	ND	NA
				Average	NA	NA		1159	130.8		NA	NA
				Std dev	NA	NA		457	51.6		NA	NA
Negative Ctrl.	NC-1	1	0	NA	ND	NA	NA	0.9	NA	NA	ND	NA
Negative Ctrl.	NC-2	1	0	NA	ND	NA	NA	<0.25	NA	NA	ND	NA
Negative Ctrl.	NC-3	1	0	NA	ND	NA	NA	<0.25	NA	NA	ND	NA
				Average	NA			0.5			NA	
				Std dev	NA			0.4			NA	
Ferrate Trt.	FT-4	2	3847	856.9	ND	NA	886.1	178	20.1	1118.3	ND	NA
Ferrate Trt.	FT-5	2	3847	856.9	ND	NA	886.1	136	15.4	1118.3	ND	NA
Ferrate Trt.	FT-6	2	3847	856.9	ND	NA	886.1	273	30.8	1118.3	ND	NA
				Average	NA	NA		195.6	22.1		NA	NA
				Std dev	NA	NA		70.1	7.9		NA	NA
pH 7 Ref.	pH-4	2	3847	856.9	ND	NA	886.1	ND	NA	1118.3	ND	NA
pH 7 Ref.	pH-5	2	3847	856.9	ND	NA	886.1	3487	393.5	1118.3	ND	NA
pH 7 Ref.	pH-6	2	3847	856.9	ND	NA	886.1	3235	365.1	1118.3	ND	NA
				Average	NA	NA		2296	308.5		NA	NA
				Std dev	NA	NA		1848	123.4		NA	NA
Ferrate Trt.	FT-7	3	32,704	7,283	297	4.1	7,532	<67	0.9	9,506	ND	NA
Ferrate Trt.	FT-8	3	32,704	7,283	212	2.9	7,532	<67[2]	0.9	9,506	ND	NA
Ferrate Trt.	FT-9	3	32,704	7,283	324	4.5	7,532	ND	0.9	9,506	ND	NA
				Average	278	3.8		67	0.9		NA	NA
				Std dev	59	0.8		0	0.0		NA	NA
pH 10.5 Ref.	pH-7	3	32,704	7,283	ND	NA	7,532	<33	0.4	9,506	ND	NA

Table 4.5 (continued)

Sample Description	Sample ID	Run #	Initial HD Added (µg/mL)	Maximum DVSO2 possible (µg/mL)	FOUND DVSO2 by LC-MS-MS (µg/mL)	Yield of DVSO2 (%)	Maximum TDG possible (µg/mL)	FOUND TDG by LC-MS-MS (µg/mL)	Yield of TDG (%)	Maximum TDGO2 possible (µg/mL)	FOUND TDGO2 by LC-MS-MS (µg/mL)	Yield of TDGO2 (%)
pH 10.5 Ref.	pH-8	3	32,704	7,283	ND	NA	7,532	<33[2]	0.4	9,506	ND	NA
pH 10.5 Ref.	pH-9	3	32,704	7,283	ND	NA	7,532	41	0.9	9,506	ND	NA
				Average	NA	NA		37	0.6		NA	NA
				Std dev	NA	NA		4	0.3		NA	NA

NA: Not Applicable

ND: Non-detect (For cases where sample triplicates contained at least one real value and one ND, the ND samples were given a value equal to the sample detection limit in order to carry out calculations, as seen with FT-7, FT-8, and FT-9 for TDG)

Note: For cases where sample triplicates contained a "<XX" or ">XX", a value of "XX" was used in order to carry out calculations, as seen with pH-7, pH-8, and pH-9 for TDG)

Footnote 4: Concentration above calibration curve. Result is estimated.
Footnote 5: Ion Ratio Out of Range

4.2.3 Run 3: HD Decontamination at Low Ferrate/Agent Ratio

4.2.3.1 Decontamination Testing and Analysis by GC-MS

Per the procedures describes in Section 3.2.2 and 3.4.5, 32.7 mg/ml of HD and a 2.7:1 mass ratio of ferrate to HD was used. Adjusting the ferrate: HD mass ration to a substoichiometric amount of ferrate forced an incomplete reaction to reveal intermediate degradation products. This was accomplished by increasing the amount of HD added to the formulation used in Run 1. All three ferrate treatments (samples FT-7, FT-8, and FT-9) turned a deep orange color upon ferrate addition, were warm to the touch, and produced off gassing when the vial cap was loosened. Within 10 min of shaking, the color had turned to brown. The observations for the non-ferrate buffer reference (samples pH-7, pH-8, and pH-9) were identical to those of Run 1 and Run 2, and hence provide three distinct reference tests at this condition with three replicates each; all were found to agree. The ferrate treatment (pH 7.0 ± 0.1) shown in Table 4.3 resulted in a relatively low decontamination level of 59 ± 1%. In contrast, the non-ferrate buffer reference (pH 6.9 ± 0.1) resulted in a substantial drop to 29 ± 9% decontamination or half the level achieved by the ferrate treatment. Supporting FS GC-MS qualitative results indicated the absence of DVSO2, dithiane, and thioxane with the exception of detecting DVSO2 in the ferrate treatment (samples FT-7, FT-8, and FT-9) (Table 4.4).

The percent HD destruction under a neutral pH (pH 7.0 ± 0.1) and a low ferrate to HD ratio of 2.7:1 wt/wt (samples FT-7, FT-8, and FT-9) was 59 ± 1%, well less than both Run 1 and Run 2 (Table 4.3). Hence the decontamination yield of ferrate is dependent upon the mole ratio and pH used, and that the best conditions, at least for HD decontamination, are those represented by Run 1.

The non-ferrate buffer (pH 6.9 ± 0.1) resulted in a lower decontamination yield than in Run 1 and Run 2 (only 29 ± 9%). The low ferrate/agent ratio of 2.7:1 apparently had too little ferrate to fully decontaminate the HD. Hence higher ratios are needed to fully decontaminate HD, greater than 2.7 but ≤ 22.5. However, as designed, the benefit of testing a very low ratio ferrate-limited condition was to

generate the condition where all the ferrate was consumed prior to the destruction of the intermediates formed during the ferrate reaction mechanism with respect to HD.

4.2.3.2 Run 3: HD Decontamination at Low Ferrate/Agent Ratio: Quantitative Product Analyses by LC-MS-MS.

The LC-MS-MS results in Table 4.5 revealed that the less desirable TDG was detected at an average concentration of 24 ± 11 µg/mL in two of the three replicates, giving an average low yield of 0.9 ± 0.0% in the ferrate treatment (samples FT-7, FT-8, and FT-9). In addition, DVSO2 was detected at an average concentration of 278 ± 59 µg/mL for an average low yield of 3.8 ± 0.8%. TDGO2 was absent (<267 µg/mL) in the ferrate treatment samples (Table 4.5). Similar to the ferrate treatment, the non-ferrate buffer (samples pH-7, pH-8, and pH-9) revealed the formation of the less desirable TDG (32 ± 7µg/mL, with an average low yield of 0.4 ± 0.1%). In addition, DVSO2 and TDGO2 were absent (<33 µg/mL and <133 µg/mL, respectively) in the non-ferrate buffer samples.

At a ferrate to HD mass ratio of 2.7:1, insufficient ferrate was present to destroy fully the normal hydrolysis product produced by the water present in the reaction mixture.

4.2.4 Summary Chemical Equations for HD Decontamination using Ferrate

Chemical equations consistent with the results for HD Run 1, Run 2, and Run 3 are assigned tentatively for the tested conditions and 60 min reaction time as follows (unbalanced):

Run 1: Ferrate Treatment (pH 10.5 target, but rising to 12.2)

$$HD + \text{xs } FeO_4^{=} \rightarrow \underset{99.1\%\text{ Yield}}{\text{low carbon number nontoxic organics (LCNNTO) and/or inorganic mineralization}}$$

Run 1: Non-ferrate buffer (pH 10.5 target, but only rising to 9.9)

$$HD + 2H_2O + 2HPO_4^{=} \rightarrow \underset{103\%}{TDG} + 2\,H_2PO_4^{-} + \underset{11\%}{\text{unreacted HD}}$$

Run 1: Water reference (pH 2.1)

$$HD + H_2O \rightarrow \underset{88\%}{TDG} + \underset{12\%}{\text{unreacted HD}} + HCl \text{ (pH 2.1)}$$

Run 2: Ferrate treatment (pH 7)

$$HD + \text{xs } FeO_4^{=} \rightarrow \underset{22\%}{TDG} + \underset{20\%}{\text{unreacted HD}} + \underset{58\ \%}{\text{(LCNNTO) and/or inorganic mineralization}}$$

Run 2: Non-ferrate buffer (pH 7 target, rising to 8.2)

$$HD + 2H_2O + 2HPO_4^{=} \rightarrow \underset{44\%}{TDG} + \underset{56\%}{\text{unreacted HD}} + 2\ H_2PO_4^{-}$$

Run 3: Ferrate treatment (pH 10.5 target, rising only to 7.0)

$$HD + \text{xs } FeO_4^{=} \rightarrow \underset{0.3\%}{TDG} + \underset{41\%}{\text{unreacted HD}} + \underset{3.8\%}{DVSO2} + \underset{54.9\ \%}{\text{(LCNNTO) and/or inorganic mineralization}}$$

4.2.5 Run 4: VX Decontamination using Ferrate

4.2.5.1 Decontaminant Testing and Quantitative Product Analyses of VX Reaction Mixtures with Ferrate by LC-MS-MS

VX was removed at a yield of 99.99 ± 0.01% for the ferrate treatment (Table 4.6, samples FT-10, FT-11, and FT-12), which can be compared to the non-ferrate buffer-only reference samples (samples pH-10, pH-11, and pH-12) that removed 65.8 ± 4.1% of the VX, and to the water reference (samples WR-4, WR-5, and WR-6) that removed 59.1 ± 9.9%. Hence ferrate removes VX in essentially quantitative yield and with high precision at test conditions, while water and pH 7 buffer solution alone leave a substantial amount of VX after one hour of reaction time.

In addition, important advantages of using ferrate emerge when considering the products formed in the decontamination reaction mixtures (Table 4.6). With the

ferrate treatment (samples FT-10, FT-11, and FT-12), ethyl methylphosphonic acid salt (EMPA) formation was detected in good reproducibility at a desirably high average concentration of 251.6 ± 3.5μg/mL, corresponding to a yield of 28.5 ± 0.4%. EMPA is a desirable stable decontamination product as it is non-toxic and forms in lieu of EA-2192, a highly toxic product often found with other decontamination chemistries (see Background). The non-ferrate buffer samples (pH-10, pH-11, and pH-12) averaged a low 75 ± 6 μg/mL EMPA, corresponding to a yield of 8.5 ± 0.7% conversion to EMPA. Water reference samples (WR-4, WR-5, and WR-6) performed similar to non-ferrate buffer, averaging 63 ± 6μg/mL with only 7.1 ± 0.7% conversion to EMPA. Ferrate treatment, non-ferrate buffer, and water alone were carried out at the same test conditions (1atm, 23°C).

Critically, EA-2192 was completely absent in the ferrate treatment samples (FT-10, FT-11, and FT-12 of Table 4.6). On the other hand, the non-ferrate buffer samples (pH-10, pH-11, and pH-12) and water reference samples (WR-4, WR-5, and WR-6) both produced some EA-2192, (14.6 ± 1.0 μg/mL with a yield of 0.86 ± 0.06% and 83.6 ± 40.9 μg/mL with a yield of 4.9 ± 2.4%, respectively).

All three test media (ferrate treatment, non-ferrate buffer, water reference) showed a small amount of formation of 2-(N, N-diisopropylamino) ethanol (DIPAE) with values of 1.9 ± 0.5 μg/mL and a yield of 0.2 ± 0.0%, 18 ± 1 μg/mL and a yield of 1.7 ± 0.1% and 16 ± 1 μg/mL with a yield of 1.5 ± 0.1%, respectively. The presence of DIPAE indicates S-C bond cleavage occurrence without amine N oxidation or N-C bond breakage. The ferrate samples showed only 10% of DIPAE as does the reference and blank. This result is desirable, as N oxidation leads to toxic compounds, but the small amount present in all samples suggests that DIPAE can be present as an impurity in the VX bulk material used for the testing. Alternatively, this nontoxic compound also can represent an intermediate in the overall reaction to LCNNTO compounds and/or mineralization.

Table 4.6. VX Decontamination Product Results by LC-MS-MS Using Ferrate

Sample Description	Sample ID	Run	Initial VX added (µg/mL)	Found VX by LC-MS-MS (µg/mL)	VX Removed by LC-MS-MS (%)	Maximum EMPA possible (µg/mL)	Found EMPA by LC-MS-MS (µg/mL)	Yield of EMPA (%)	Maximum DIPAE possible (µg/mL)	Found DIPAE by LC-MS-MS (µg/mL)	Yield of DIPAE (%)	Maximum EA-2192 possible (µg/mL)	Found EA-2192 by LC-MS-MS (µg/mL)	Yield of EA-2192 (%)
Ferrate Trt	FT-10	4	1903	0.345	99.98	883.4	247.6	28.0	1034.1	<0.15	0.015	1703.4	ND	0
Ferrate Tr.	FT-11	4	1903	<0.03	>99.99	883.4	252.9	28.6	1034.1	<0.15	0.015	1703.4	ND	0
Ferrate Tt.	FT-12	4	1903	<0.03	>99.99	883.4	254.2	28.8	1034.1	<0.15	0.015	1703.4	ND	0
			Average→	0.135	99.99		251.6	28.5		<0.2	0.015		ND	0
			Std Dev→	0.182	0.005		3.5	0.4		0.0	0.0		NA	NA
pH 7 Ref	PH-10	4	1903	561.6	70.49	883.4	<75	8.5	1034.1	17.04	1.6	1703.4	13.95	0.82
pH 7 Ref	PH-11	4	1903	688.2	63.84	883.4	77.1	8.7	1034.1	19.38	1.9	1703.4	15.78	0.93
pH 7 Ref	PH-12	4	1903	703.2	63.05	883.4	80.4	9.1	1034.1	16.71	1.6	1703.4	14.07	0.83
			Average→	651.0	65.80		78	8.8		17.71	1.7		14.60	0.86
			Std Dev→	77.8	4.09		2.7	0.3		1.5	0.1		1.0	0.06
Water Ref	WR-4	4	1903	563.4	70.40	883.4	<75	8.5	1034.1	14.52	1.4	1703.4	38.28	2.25
Water Ref	WR-5	4	1903	911.1	52.13	883.4	<75	8.5	1034.1	16.98	1.6	1703.4	118.02	6.93
Water Ref	WR-6	4	1903	862.2	54.70	883.4	<75	8.5	1034.1	15.90	1.5	1703.4	94.35	5.54
			Average→	245.9	59.08		75	8.5		15.80	1.5		83.55	4.90
			Std Dev→	188.2	9.89		0.0	0.0		1.23	0.1		40.95	2.40

ND: Non-detect
NA: Not Applicable

Std Dev: Standard Deviation
Note: For cases where sample triplicates contained a "<XX" or ">XX", a value of "XX" was used in order to carry out calculations, as seen with FT-10, FT-11, and FT-12 for VX)

The VX samples treated with ferrate contained about 15% of the amount of DIPAE shown to be in the buffer and water reference systems. This quantified difference indicates that ferrate significantly decontaminates this component of VX mixtures.

4.2.6 Chemical Equations for VX Decontamination using Ferrate

Based on the results given above, the unbalanced chemical reaction for VX decontamination using ferrate is summarized by the following equation (unbalanced):

Run 4: Ferrate Treatment of VX at pH 7

$$VX + xs\ FeO_4^{=} \rightarrow \underset{28.5\%}{EMPA} + \underset{0.2\%}{DIPAE} + \underset{71.3\ \%}{LCNNTO} \text{ and/or inorganic mineralization}$$

(with no generation of EA-2192, < 0.002%)

4.2.7 QA/QC Results of FS GC-MS for HD Analyses

Calibration curves were linear for HD with correlation coefficients (r^2) of 0.995 or greater for all analysis sequences. All check standards fell within the requirements ± 30%. The standards for HD were run consecutively at the beginning of the run for HD. In addition, all spike controls exceeded the specifications of ± 20% of the expected value.

In order to keep the sample concentrations within the limits of the calibration curves, dilutions of 1:10, 1:25, 1:100, or 1:1000 were necessary on select samples (Table 4.7). Samples diluted 1:10 had 100 µL of sample (using a 100 µL syringe) added to 900 µL isooctane (using a 1.0 mL syringe). Samples diluted 1:25 had 40 µL of sample (using a 50 µL syringe) added to 960 µL isooctane (using a 1.0 mL syringe). Samples diluted 1:100 had a 10 µL sample (using a 10 µL syringe) added to 990 µL isooctane. Samples diluted 1:1000 were first diluted 1:10 with 100 µL added to 900 µL solvent and then diluted 1:100.

Table 4.7. HD FS GC-MS Sample Dilutions

Sample ID	Agent	Dilution(s)				
		None	1:10	1:25	1:100	1:100
FT1 - FT3	HD	✓	—	—	✓	—
FT4 - FT6	HD	✓	—	—	✓	✓
FT7 – FT9	HD	✓	✓	—	✓	—
PH1 – PH3	HD	—	—	—	✓	—
PH4 – PH6	HD	—	—	—	—	✓
PH7 – PH9	HD	—	—	—	✓	
WR1 – WR3	HD	—	✓		✓	—
NC1 – NC3	None	✓	—	—	—	—

4.2.8 QA/QC Results of LC-MS-MS

4.2.8.1 HD Analysis

All water blanks analyzed by the analytical laboratory for DVSO2, TDG, and TDGO2 throughout the various sequences that encompassed the ferrate treated samples, pH-reference buffer samples, and water reference samples were clean and demonstrated no carryover for these target analytes (Table 4.8).

Table 4.8. LC-MS-MS HD Water Blanks

	DVSO2	TDG	TDGO2
IDL (ng/mL)	25	25	100
	Concentration (ng/ml)		
Water Instrument Blank	ND	ND	ND

IDL: Instrument Detection Limit
ND: Not Detected

Calibration curves were linear for DVSO2, TDG, and TDGO2 with r^2 of about 0.99 for most analysis sequences with two exceptions. TDGO2 had an r^2 value of 0.966 for the calibration curve analyzed on July 27, 2005, and 0.974 for the calibration curve analyzed on August 02, 2005. Analytical results should not be impacted since TDGO2 was not detected in any of the samples associated with these calibration curves. Calibration curves were used only to demonstrate linearity and not to quantify the analytical results. All quantitations were based on the bracketing Instrument Calibration and Verification (ICV) standards.

Most ICVs exhibited acceptable recoveries (within 40-60%) for DVSO2, TDG, and TDGO2 throughout the various analysis sequences that encompassed all samples and dilutions and ranged from ~46-126%, with three exceptions. The ICV recovery was biased low at 33% for TDG in the ending ICV associated with the analysis of samples pH-1, pH-2, pH-3, and pH-4 on July 20, 2005, biased slightly high (163%) for TDGO2 in the initial ICV associated with the analysis of samples pH-5, pH-6, pH-7, pH-8, and pH-9 on July 27, 2005, and biased high (183%) in the ending ICV associated with the reanalysis of samples pH-5, pH-6, WR-1, WR-3, and NC-1 on August 02, 2005. Analytical results for TDG in the above samples can be biased high. Analytical results for TDGO2 should not be impacted since TDGO2 was not detected in any of these samples.

Post-spiking was added to a representative ferrate treated sample (FT-9) with a solution containing DVSO2, TDG, and TDGO2 so that the concentration in the final extract of 5 X the IDL was used to confirm the presence or absence of these target analytes in the native sample (Table 4.9). The percent recoveries of the target analytes in the post-spiked sample (corrected for any native contribution) were acceptable. In order to keep the sample concentrations within the limits of the calibration curves, dilutions of 1:10, 1:533, 1:1333, 1:2667, and 1:6667 were necessary on some samples (Table 4.10).

Table 4.9. LC-MS-MS HD Post-Spike

	DVSO2	TDG	TDGO2
Post-Spiking Level (ng/mL)	125	125	500
Sample	% Recovery		
FT-9 Spike	112	112	112

Table 4.10. LC-MS-MS HD Sample Dilutions

Sample Name	Spiked Conc (ppm)	µL Sample Used	Final Volume (mL)	Dilution Factor	Notes
FT-1	1000	18.75	10.0	533	—
FT-2	1000	18.75	10.0	533	—
FT-3	1000	18.75	10.0	533	—
FT-4	1000	18.75	10.0	533	—
FT-5	1000	18.75	10.0	533	—
FT-6	1000	18.75	10.0	533	—
FT-7	5000	3.75	10.0	2667	—
FT-8	5000	3.75	10.0	2667	—
FT-9	5000	3.75	10.0	2667	—
PH-1	1000	7.5	10.0	1333	—
PH-2	1000	7.5	10.0	1333	—
PH-3	1000	7.5	10.0	1333	—
PH-4	1000	7.5	10.0	1333	—
PH-5	1000	7.5	10.0	6667	1:5 dilution made after initial analysis
PH-6	1000	7.5	10.0	6667	
PH-7	5000	7.5	10.0	1333	1:5 dilution made prior to final dilution
PH-8	5000	7.5	10.0	1333	
PH-9	5000	7.5	10.0	1333	
WR-1	1000	7.5	10.0	6667	1:5 dilution made after initial analysis
WR-2	1000	7.5	10.0	1333	—
WR-3	1000	7.5	10.0	6667	1:5 dilution made after initial analysis
NC-1	0	10	1.0	10	—
NC-2	0	10	1.0	10	—
NC-3	0	10	1.0	10	—

4.2.8.2 VX Analysis

All water blanks analyzed by the analytical laboratory for EMPA, DIPAE, EA-2192, and VX throughout the various sequences that encompassed the ferrate treated samples, pH-reference buffer samples, and water reference samples were considered to be clean and demonstrated no carryover for these target analytes (particularly EA 2192 and VX) above 1% of the highest level calibration standard analyzed in the sequence (Table 4.11).

Table 4.11. LC-MS-MS VX Water Blanks

	EMPA	DIPAE	EA-2192	VX
IDL (ng/mL)	25	5.0	1.0	5.0
	Concentration (ng/ml)			
Water Instrument Blank	ND	ND	ND	ND

IDL: Instrument Detection Limit
ND: Not Detected

Calibration curves were linear for EMPA, DIPAE, and VX with r^2 of > 0.99 for all analysis sequences. Calibration curve for EA-2192, however, was quadratic. Calibration curves were used only to demonstrate linearity and not to quantify the analytical results. All quantitations were based on the bracketing ICV standards. ICVs exhibited acceptable recoveries (within 40-160%) for EMPA, DIPAE, EA 2192, and VX throughout the various analysis sequences that encompassed all field samples, with recoveries ranging from ~69-31%.

Post-spiking was added to representative pH-reference and water reference samples (pH-12 and WR-6) with a solution containing EMPA, DIPAE, EA-2192, and VX so that the concentration in the final extract of 5X the IDL was used to confirm the presence or absence of these target analytes in the corresponding native samples (Table 4.12). The percent recoveries of the target analytes in the post-spiked samples (corrected for any native contribution) were acceptable for EMPA, DIPAE, and EA 2192, but were meaningless for VX; the concentration of VX in the native samples was significantly higher than the post-spiked concentration. All VX decontamination samples were diluted 50-fold prior to receipt at the analytical laboratory. In order to keep the sample concentrations within the limits of the calibration curves, additional dilutions of 1:50, 1:100, and 1:400 were necessary on some samples (Table 4.13).

Table 4.12. LC-MS-MS HD Post-Spike

	DVSO2	TDG	TDGO2
Post-Spiking Level (ng/ml)	125	125	500
Sample	% Recovery		
FT-9 Spike	112	112	112

Table 4.13. LC-MS-MS VX Sample Dilutions

EMPA, DIPAE, and EA-2192				
Sample Name	**Spiked Conc (ppm)**	**µL Sample used**	**Final Volume (mL)**	**Dilution Factor**
PH-10	63.4	10	1.0	100
PH-11	63.4	10	1.0	100
PH-12	63.4	10	1.0	100
WR-4	63.4	10	1.0	100
WR-5	63.4	10	1.0	100
WR-6	63.4	10	1.0	100
VX				
Sample Name	**Spiked Conc (ppm)**	**µL Sample used**	**Final Volume (mL)**	**Dilution Factor**
PH-10	63.4	5	2.0	400
PH-11	63.4	5	2.0	400
PH-12	63.4	5	2.0	400
WR-4	63.4	5	2.0	400
WR-5	63.4	5	2.0	400
WR-6	63.4	5	2.0	400
DIPAE, EA-2192, and VX				
Sample Name	**Spiked Conc (ppm)**	**µL Sample used**	**Final Volume (mL)**	**Dilution Factor**
FT-10	63.4	NA	NA	1
FT-11	63.4	NA	NA	1
FT-12	63.4	NA	NA	1
EMPA				
Sample Name	**Spiked Conc (ppm)**	**µL Sample used**	**Final Volume (mL)**	**Dilution Factor**
FT-10	63.4	20	1.0	50
FT-11	63.4	20	1.0	50
FT-12	63.4	20	1.0	50

The results of the thermal stability testing of potassium ferrate indicate that K_2FeO_4 TG crystals are quite stable at the conditions specified by AR 70-38 Sec. II, Table 2-2 (Storage and Transit Conditions). Thermal stability has been a serious barrier to commercialization of the otherwise desirable peroxide decomposition chemistry. It is surmised that the ferrate ion, $FeO_4^{=}$, tetrahedral (Td) structure, being almost identical to that of the highly symmetrical sulfate ion, $SO_4^{=}$, figures into causing the observed high solid state stability of ferrate ion. Like potassium sulfate, potassium ferrate is extremely water soluble and dissolves rapidly, enabling a

strongly reacting decontamination agent to be readily prepared at the point of use, with only a small amount of water needed, and from a stable solid product.

Although the development of a decontamination protocol was not within the scope of this work, it is offered that an effective procedure to decontaminate HD or VX, and most likely many other CB agents, with ferrate is to treat the contaminated surface with a sufficient excess of ferrate in two steps at ambient temperature;

- Step 1: Apply ferrate as a powder or a thin layer of liquid (Part 1).
- Step 2: Apply a buffer/PTC mixture as a powder or water mist (Part 2).
- Let stand until discoloration occurs (from purple to orange-brown). (Standing time has yet to be determined precisely, and determining it was out-of-scope for the current project).
- Rinse to non-hazardous sewer (optional), or sweep up to non-hazardous waste disposal (optional), or let stand, depending on the nature of the surface contaminated.

Specifics, including order of Steps 1 and 2, could be developed in future testing (see Recommendations). This procedure allows the pH to drift downwards during the treatment but the pH is always at mild values to prevent corrosion of the surface being treated, and to provide a full range of oxidation strength and other ferrate-driven decontamination reactions to occur. The buffer, or equivalent, provides the means to prevent the pH from entering a hazardous or corrosive region for the surfaces being decontaminated.

5 CONCLUSIONS

The above results allow a number of key conclusions to be drawn regarding the use of ferrate as a decontamination reagent formulation against HD and VX:

- Ferrate was found to be effective in the quantitative (>99% for HD and 99.99% for VX) decontamination of these agents when used in the manner of Run #1 and as Formulation 1. Qualitative indications, based on rate of ferrate color disappearance, suggest that the decontamination reaction is fast.

- Critically, this decontamination is accomplished while not forming toxic organic products found with other decontamination chemistries. Such toxic products were formed in the reference and blank cases.

- Apparently, the toxic products that form with conventional hydrolysis treatments of water and alkaline pH, in the absence of ferrate, either do not have time to form when ferrate decontamination reagent is present, or do form and are destroyed rapidly by ferrate, or both occur.

- Ferrate accomplishes substantial decontamination of HD and VX into small low carbon number non-toxic organic (LCNNTO) compounds and/or inorganic salts (mineralization) when a large ferrate/agent ratio (e.g., 22.5:1 or 45:1, respectively) is used. Essentially full oxidation of agents is reasonable thermodynamically given the high oxidation potential and kinetic reactivity of ferrate and given the sufficiently high ferrate/agent ratio used.

- Potassium ferrate, the active decontamination component, exhibited good thermal stability. After 98 days of isothermal storage at 71°C and 82 days of cyclic temperature storage (up to 71°C) respectively, 90% and 93% of the original ferrate crystals remained intact.

6 RECOMMENDATIONS

With the positive concept validation results presented in this report, further testing and product development work is warranted towards the development of ferrate for broad spectrum CBD use. Recommended future work includes:

- Determine the specific low carbon number nontoxic organics (LCNNTO) degradation products of HD and VX by determining molecular and elemental mass balances. Much of the agent mass appears to have been reduced to LCNNTO (e.g., acetate and others) and possibly minerals (carbonate, sulfate, phosphonate chloride, phosphates, and nitrate ions). Such data could be collected using ion chromatography of archived samples from this project.

- Establish the kinetics of agent decontamination using ferrate by measuring the rate of CWA decontamination vs. time in the range of 10 min to 60 minutes. Such data would be used to refine the composition of the ferrate-formulated product and the time needed for complete decontamination to occur. These data would be determined at a wide range of temperatures so that exposure time recommendations could be made for varying environmental factors. It would be useful if the purple color fading was found to be synchronized with disappearance of agent.

- Optimize the ferrate decontamination formulation packaging, and use protocols. Ultimately a fully formulated packaged product is required in large quantities for worldwide distribution. This recommended work would involve DoD Laboratories with the special skills to develop MIL-Spec packaging. The properly formulated and packaged product would include a task to develop a simplified use protocol (minimum steps, equipment, water needed, etc.).

- Evaluate ferrate decontamination performance with respect to surface decontamination testing using coupon testing.

- Determine the preferred use protocol(s) for decontamination of surfaces, equipment, and personnel with ferrate.

- Conduct a comprehensive test with the refined ferrate formulation on representative biological agents.

7 REFERENCES

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Appendix A

Standard Operating Procedure for Potassium Ferrate(VI) Decontamination Testing for HD and VX

Key words: Potassium Ferrate (VI)

STANDARD OPERATING PROCEDURE FOR POTASSIUM FERRATE(VI) DECONTAMINATION TESTING

Originated by: _____ Date: _____
Principal Investigator
Hazardous Materials Research Center

Reviewed by: _____ Date: _____
Program Manager
Hazardous Materials Research Center

Approved by: _____ Date: _____
Chemical Hygiene Officer
BSTI Safety, Health and Emergency Response

Approved by: _____ Date: _____
Department Manager
Hazardous Materials Research Center

Approved by: _____ Date: _____
CET QA Manager
Hazardous Materials Research Center

Distribution List:
Quality Assurance
SOP Manuals

Effective Date: _____

Battelle
Hazardous Materials Research Center
505 King Avenue
Columbus, Ohio 43201

I/II. Scope/Purpose

The objective of this test is to determine the decontamination efficacy of a potential decontaminant, Potassium Ferrate(VI) or "ferrate". Chemical agents VX and HD will be added to reaction vessels containing a unique Ferrate Formulation (FF), sealed and then shaken for up to 90 minutes. Reaction vessels will be pulled and extracted to determine decontamination efficacy under both wet and dry conditions. This task will be performed in the Hazardous Materials Laboratory, at Battelle Memorial Institute's HMRC.

III. References

Standard Operating Procedure: General Provisions for Handling Chemical Surety Material in the Hazardous Materials Research Facility (SOP HMRC-II-001)

Standard Operating Procedure: Analysis of Solutions Containing GA, GB, GD, HD, and VX by GC (SOP HMRC-IV-013)

Standard Operating Procedure: 4X Materials Proof of Decon (SOP HMRC-III-007)

Standard Operating Procedure: Chemical Agent Decontamination and the Collection and Disposal of Waste at Battelle's HMRC (SOP HMRC-I-011)

Standard Operating Procedure for Packaging, and Transport of Dilute CA (RDS) (SOP HMRC III-028-01)

IV. Definitions

See SOP HMRC-II-001, Sections IV.A through IV.W.

V. Procedure

A. Hazards

Operators will be thoroughly familiar with the hazards associated with the following:

Agents: VX and HD

Decontaminants: Minimum 5.0% bleach for HD, 10% HTH for VX.

Test Chemicals: Potassium Ferrate(VI), Solid Potassium Phosphate (monobasic) buffer, Potassium Hydroxide (KOH), and Aliquat® 336 (Phase Transfer Catalyst:PTC).

NOTE: MSDS for all test chemicals are on file.

Solvents: Isooctane and Hexane.

B. Safety Precautions

All Safety and emergency requirements outlined in HMRC-SOP-II-001, Sections V.B and V.C will be followed when handling CSM.

C. Equipment

In addition to the equipment listed in HMRC-II-001, the following equipment will be required:

Hand motion shaker
20ml scintillation vials with Teflon lined cap
15ml centrifuge tubes with caps
4ml glass vials with Teflon lined cap
100ml glass jars with Teflon lined cap
Pasteur Pipettes
Top loading balance
GC vials and caps
5ml and 10ml disposable glass pipets
Portable pipet-aid
Vortex mixer
100ul and 500ul Hamilton syringe, blunt needle stepper
Eppendorf pipet (100ul – 1000ul)
pH meter with probe

D. General Instructions

Ferrate will be prepared at Battelle, 505 King Avenue laboratories. The preparation of the Ferrate will not be outlined in this SOP. The Ferrate powder along with a monobasic buffer and phase transfer catalyst (PTC) will be pre-measured and added to the appropriate scintillation vials (reaction vessels) and will constitute a unique FF. The FF components will be prepared at King Avenue laboratories and shipped to the HMRC. Each 20ml scintillation vial will either be left dry or 3mL of deionized (DI) water will be added. Next, neat liquid agent will be added to all vials. After all vials are spiked, they will be vortexed for 10 second intervals then placed on a shaker for up to 90 minutes. After the appropriate time, vials will be removed from the shaker and the entire contents of the vials extracted with a series of two (2) 5ml isooctane extracts. Finally, an aliquot of the isooctane extract will be transferred to a GC vial for analysis.

E. Specific Instructions

1. Prepare the work area and set up the hood in accordance with HMRC-II-001, Section V.E.2 and V.E.3.

   Note: Be sure Decon buckets are placed on a spill tray covered with absorbent paper.

2. Select one (1) vial of **Component A** supplied from Battelle Columbus Operation (BCO). Record the reference # of the vial in the LRB.

3. Don minimum CSM clothing in accordance with SOP HMRC-II-001, Section V.E.4.

of ferrate in samples is still purple, return all vials to the shaker for another 30 min. If ferrate sample color still is purple after this second shake, proceed to step 21.

Note: Possible colors: brown, purple, orange, colorless, and white. Record presence/absence of solids, turbidity, particulates, gel, etc. in LRB.

21. Carefully remove the cap of the completed vial, place it on a plastic backed wipe and take a pH measurement of each vial and note in LRB. In order to avoid cross contamination between samples, hold the pH probe over the decon bucket and rinse probe tip three times with DI water after each reading, and return probe to pH 7 buffer for storage.

    Note: pH probe should be calibrated at pH 4, 7, and 10 at the start of each day and recorded in the LRB.

22. **If working with HD**: To the completed vial, transfer in 5.00mL of isooctane using a 5mL graduated pipette. Recap the vial, secure tightly and vortex for approximately 30 seconds. Following vortexing, allow approximately 15 minutes for the sample to phase.

23. **If working with VX:** Vortex the completed vial for approximately 10 seconds and immediately transfer precisely 2.00ml, using a 5mL graduated pipet into a vial for later use. With the remaining volume (approximately 1.0ml) of the vial, transfer in 5.00mL of isooctane using a 5mL graduated pipette. Recap the vial, secure tightly and vortex for approximately 30 seconds. Following vortexing, allow approximately 15 minutes for the sample to phase break.

    Note: If running VX positive controls: To the completed vial, adjust the pH to approximately 7-7.5 using KOH. Note initial and final pH of solution and the approximate number of KOH drops it took to reach the final pH. Next, transfer in 5.00mL of isooctane using a 5mL graduated pipette. Recap the vial, secure tightly and vortex for approximately 30 seconds. Following vortexing, allow approximately 15 minutes for the sample to phase break.

24. Place the completed vial into a tray covered in brown craft paper. With the vial remaining in the tray, cover the cap with a wipe and remove the cap, being careful not to disturb the phases. Remove the solvent layer (top layer) from the vial to a clean centrifuge tube using a Pasteur pipet (vial may be removed from tray at this time to aid in visualization of phases). Perform a second extraction and combine with the first extract in the centrifuge tube. Cap and securely tighten.

    Note: If working with HD: Following the second extraction, the remaining contents of the vial, approximately 3ml will be retained in the original vial and submitted to BCO for analysis. All BCO samples will be RDS and submitted to the Analytical Chemistry Group at 505 King Ave per HMRC SOP-III-028-01. For positive controls, the 3ml will be retained in the original vial and archived in a freezer.

    If working with VX: Following the second extraction, the remaining contents of the vial, approximately 1ml will be retained in the original vial and archived in either a refrigerator or

freezer. For positive controls, approximately 3ml will be retained in the original vial and archived in either a refrigerator or freezer.

25. By this Step, both extractions from Step 24 should be in one (1) centrifuge tube with a total volume of approximately 10ml.

26. **This step is skipped if working with HD or VX spike controls.** If needed, with the vial from step 23 containing approximately 2ml, a 30X dilution will be carried out to ensure contents are <100ug/ml. Transfer 200ul into a clean vial using a Eppendorf pipet. In addition, transfer 5.8ml of DI water into the same vial, using a 10ml disposable pipet. Cap and securely tighten. Vortex for 10 sec. This vial now containing approximately 6ml will be submitted to BCO for analysis. All BCO samples will be RDS and submitted to the Analytical Chemistry Group at 505 King Ave per HMRC SOP-III-028-01. The original vial now containing approximately 1.8ml will be retained and archived in a freezer.

    Note: This step is required only if VX spike controls reveal poor extraction efficiency from water.

27. At the conclusion of the test, unplug the shaker from the wall outlet. Check to see that none of the vials leaked any material into the shaking tray. If it is determined that one or more of the vials leaked, the shaking tray will be rinsed either with 5.0% bleach for HD or 10% HTH for VX.

28. Transport all appropriate vials and centrifuge tubes from steps 19, 24, and 25 to analytical.

29. Once in analytical, if needed, the centrifuge tubes may be spun down for approximately 5 minutes.

30. Using a Pasteur pipet, transfer approximately 1ml of the solvent layer (top layer) from the centrifuge tube to a GC vial, securely cap, and analyze by GC/MS. Transfer the remaining solvent layer (approximately 9ml) to a clean vial, securely cap and archive in a freezer.

F. Decontamination

1. All HTH waste will be separated into solids and liquids. The solid waste will be placed into the non-regulated hazardous waste drum. After completing the decontamination, the liquid waste will be placed in the HML pouring station sink that drains to the holding tanks. Isooctane and other organic solvents will be placed in a RCRA solvent waste drum.

2. All tools, contaminated equipment, and the work area will be cleaned following procedures detailed in SOP HMRC-II-001, Section V.E.17 and 18.

3. The shaker and vortex will be allowed to air purge, be double bagged, and proof of decon (POD) performed following procedures detailed in SOP HMRC – III-007-07. Upon successful 3X POD, the vortex and shaker will be stored for further use.

G. Emergency Procedures

Any actions necessitated by emergencies will be conducted as described in SOP HMRC-II-001, Section V.E.19 through V.F and V.G.

H. First Aid / Self Aid

If physical injuries occur, first aid or self aid will be administered and the nurse located on site will be called. If extensive injury results, the ambulance will be summoned by dialing 911. If the telephone line is inoperable or busy, the two-way radio located outside the analytical lab will be used.

SOP NUMBER AND TITLE: HMRC-X-147-01 "Potassium Ferrate (IV) Decontamination Testing"
REVISION NUMBER AND DATE:00 6/13/05
ANALYSIS COMPLETED BY: Dave Stitcher, Brian Blackstone, Fred Moore, and Adam Reuther

HAZARD ANALYSIS

CATEGORY	"WHAT IF"	CONSEQUENCES	PROTECTION	RAC[1]
V. E. 2	Decon bucket leaks	Potential for decon solution to come out of hood and burn operator.	Place a spill tray down over entire working surface and cover with absorbent paper.	III-D(III-E)
V. E. 2	Placement of syringe used to spike vials contaminates operators gloves	Personnel not protected from hazards. Potential for agent exposure.	Ensure a large enough glass pyrex pan is setup in hood.	III-C(III-E)
V. E. 12	Pressure is built up in vial during vortexing.	Contents of vial could spew, resulting in personal exposed to splash hazard.	Place vial in tray while decapping and cover cap with plastic back wipe.	III-A(III-D)

[1] Risk Assessment Code: I - IV represents the severity of the hazard, with "I" as catastrophic and "IV" as negligible.
A - E represents the probability of the hazard occurring, with "A" as frequent and "E" as improbable

Appendix B

Sample Handling and Analysis Flow Scheme for HD and VX Decontamination Test Samples

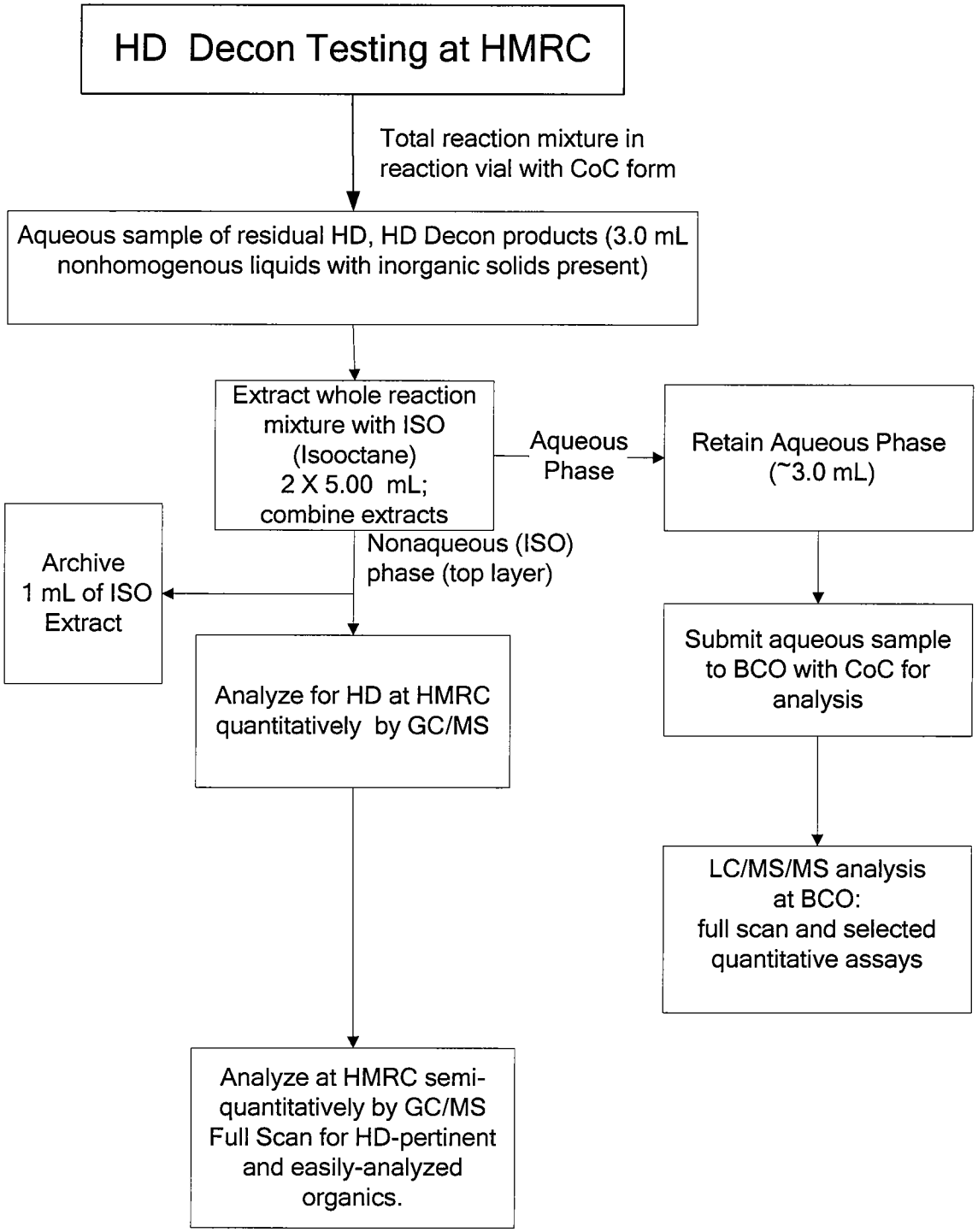

Figure B.1. Sample Analysis Flow Scheme for HD Decontamination

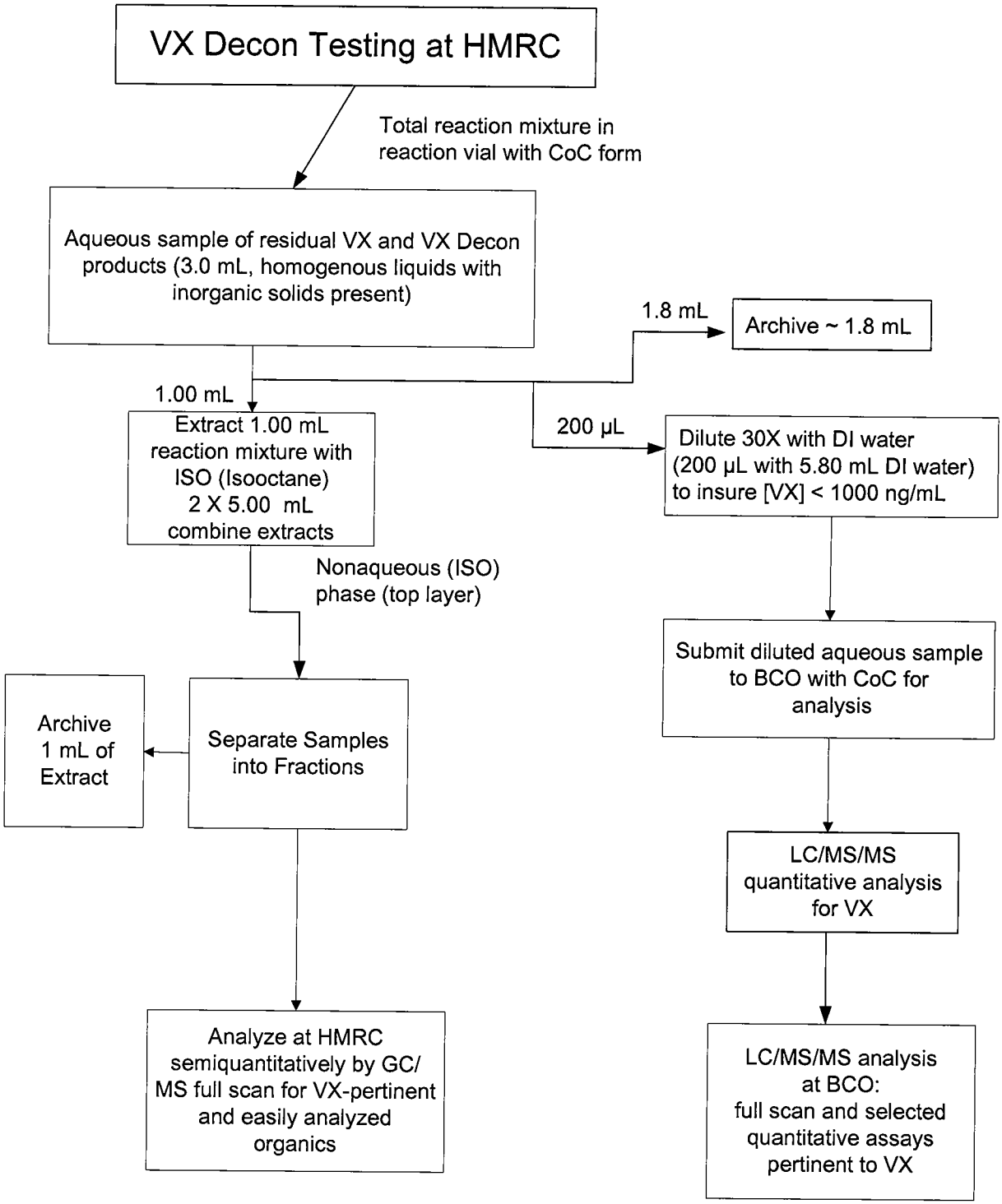

Figure B.2. Sample Handling and Analysis Flow Scheme for VX DECONTAMINATION Reaction Mixtures

Appendix C

Analysis of Reaction Products of HD Decontamination Testing by Ferrate using LC-MS-MS

1.0 Scope and Application

This analysis method is used to determine the concentration of divinyl sulfone, thiodiglycol, and bis(2-hydroxy ethyl) sulfone in water or other reverse-phase liquid chromatography compatible solvent. High Pressure Liquid Chromatography (HPLC) is coupled with a PE SCIEX Triple Quadrapole Mass Spectrometer (APCI+MS-MS), resulting in a highly selective and sensitive analysis technique.

Table C. 1. m/z and Calibration Levels for Each Target Analyte

Analyte	Parent Ion (m/z)	Daughter Ions (m/z)	Approx. Retention time (min)	Calibration levels				
				1X ng/mL	2X ng/mL	5X ng/mL	15X ng/mL	30X ng/mL
Divinyl Sulfone	119	93, 75	6.9	25	50	125	375	750
Thiodiglycol	123	105, 45	5.6	25	50	125	375	750
Bis(2-hydroxy ethyl) sulfone	155	109, 45	2.9	100	200	500	1500	3000

2.0 Apparatus and Materials

- Mass Spectrometer: PE Sciex API III+ Triple Quadrapole Mass spectrometer with a Vaporjet / APCI source.
- HPLC: 2 Shimadzu LC-10AD HPLC pumps or equivalent; Alcott 708 autosampler or equivalent with 200 µL injection loop
- Column: Restek C18 ultra aqueous 150 x 4.6 mm reverse-phase analytical column or equivalent
- Mobile Phase: A= Milli-Q water containing 2 mM each ammonium formate and formic acid; B=HPLC grade methanol containing 2 mM each ammonium formate and formic acid (See Table C-2 for pump conditions).

Table C. 2. HPLC Pump Gradient Time Table

Time (min)	%A	%B	Flow (mL/min)
0.00	95	5	1.0
2.00	95	5	1.0
7.00	5	95	1.0
10.00	5	95	1.0
10.01	95	5	1.0
15.00 (STOP)	95	5	1.0

- Calibration Standards: Calibration standards prepared in water at the concentrations found in Table C.1 can be used for up to 1 month if stored at -20 $\pm$ 3°C.

3.0 Sample Preservation

To reduce the rate of hydrolysis during standing, maintain prepared samples at -20 $\pm$ 3°C prior to analysis.

4.0 Procedure

- Mass Calibration Verification: Before each batch analysis, the analyst will verify the mass calibration of Quadrapole 1 (Q1) and Quadrapole 3 (Q3). The mass verification will be handled by introducing a compound of a mass known to be within the range of the target analytes to the plenum of the mass spectrometer. A Q1 and Q3 scan will be performed of the mass of the test compound. The measured mass must be within 0.2 amu of the nominal mass of the test compound with the peak measuring between 0.5 and 1.0 amu at full width/half maximum.

- Analyte Calibration: A calibration curve of at least three points (five recommended) will be analyzed with an $r^2 \geq 0.97$. Subsequent analyses can be performed using a low standard to verify detection limits and the rolling quantitation standards.

5.0 Quantitative Analysis

- Rolling Quantitation: A mid-level standard (15X recommended) injected at least once per 15 injections serves to correct for sensitivity loss over time by averaging the mid-level standards (see below) and using that value to determine a response factor.

- Rolling Quantitation formula:

$$[(L41+L42)/2]/Ci=RFi$$

where:
L41= area of first bracketing standard
L42= area of second bracketing standard
Ci= concentration of analyte in standard
RFi= response factor of analyte i
Then:
X=Ai/ RFi

X= concentration of analyte i, in the analyzed sample
Ai= area of analyte i.

- Interferences: When analyzing noninterfering matrices, the analyst should quantitate on the parent/daughter ion that appears to give the best signal to noise ratio unless other factors indicate that another ion could give more accurate results. If interferences appear in the matrix, then two ions for each compound should be monitored, allowing the standard ratio to be compared to the sample ratio.

- Dilutions: If an analyte is detected at a level calculated to be above the calibration range, the sample will be diluted and reanalyzed.

APPENDIX D

Analysis of the Nerve Agent VX and VX Reaction Products by LC-MS-MS

1.0 Scope and Application

This analysis method is used to determine the concentration of VX, EA-2192, diisopropyl amino ethanol, and ethyl methyl phosphonate in water or other reverse-phase liquid chromatography (LC) compatible solvent. High Pressure Liquid Chromatography (HPLC) is coupled with a PE SCIEX Triple Quadrapole Mass Spectrometer (ESI-MS-MS), resulting in a highly selective and sensitive analysis technique (Table D.1).

Table D.1. m/z and Calibration Levels for Each Target Analyte

Analyte	Parent Ion (m/z)	Daughter Ions (m/z)	Approx Retention time (min)	Calibration levels				
				1X (ng/mL)	5X (ng/mL)	15X (ng/mL)	30X (ng/mL)	45X (ng/mL)
VX	268	128, 86	30	5	25	75	150	225
EA-2192	240	128, 86	12.5	5	25	75	150	225
Diispropyl amino Ethanol	146	86, 44	22	5	25	75	150	225
Ethyl methyl phosphonate	125	97, 79	2.3	5	25	75	150	225

2.0 Apparatus and Materials

- Mass Spectrometer: PE Sciex API III + triple quadrapole mass spectrometer with an electrospray source and heated nebulizer (Turbolon Spray®); see Table D-2.

Table D.2. Electrospray Source Conditions

Nebulizer	0.6 L/min @ 40 psi UHP nitrogen
Turbolon Spray®	7.0 L/min @ 350°C UHP nitrogen
Curtain Gas	0.6 L/min UHP nitrogen

- HPLC: 2 Shimadzu LC-10AD HPLC pumps or equivalent; Alcott 708 autosampler or equivalent with 50 µL injection loop
- Column: Restek PFP propyl 150 X 2.1 mm reverse-phase analytical column or equivalent

- Mobile Phase: A= 98 % Milli-Q water, 2% HPLC grade acetonitrile; B= 90% HPLC grade acetonitrile/10% Milli-Q water, containing 2 mM each ammonium formate and formic acid (See Table D.3 for pump conditions)

Table D.3. HPLC Pump Gradient Time Table

Time (min)	A%	B%	Flow (mL/min)
0.00	100	0	0.2
4.00	100	0	0.2
14.00	0	100	0.2
35.00	0	100	0.2
35.01	100	0	0.2
45.00 (STOP)	100	0	0.2

- Calibration Standards: Calibration standards prepared in water from CAC solutions at the concentrations found in Table D.1 can be used for up to 1 week if stored at -20 $\pm$ 3°C.

3.0 Sample Preservation

To reduce the rate of hydrolysis, maintain prepared samples at -20 $\pm$ 3°C prior to analysis.

4.0 Procedure

- Mass Calibration Verification: Before each batch analysis, the analyst will verify the mass calibration of Quadrapole 1 (Q1) and Quadrapole 3 (Q3). The verification will be handled by introducing a compound of a mass known to be within the mass range of the target analytes to the plenum of the mass spectrometer. A Q1 and Q3 scan will be performed of the mass of the test compound. The measured mass must be within 0.2 amu of the nominal mass of the test compound with the peak measuring between 0.5-1.0 amu at full width/ half maximum.

- Analyte Calibration: A calibration curve of at least three points (five recommended) will be analyzed with an $r^2 \geq 0.97$. Subsequent analyses can be performed using a low standard to verify detection limits and the rolling quantitation standards.

5.0 Quantitative Analysis

- Rolling Quantitation: A mid-level standard (15X recommended) injected at least once per 15 injections serves to correct for sensitivity over time by averaging the

mid-level standards (see below) and using that value to determine a response factor.

- Rolling quantitation formula:

$$[(L4_1+L4_2)/2]/Ci=RF_i$$

where:
$L4_1$= area of first bracketing standard
$L4_2$= area of second bracketing standard
Ci= concentration of analyte in standard
RF_i= response factor of analyte i
Then:
$X=A_i/RF_i$
X= concentration of analyte i, in the analyzed sample
A_i= area of analyte i.

- Interferences: When analyzing noninterfering matrices, the analyst should quantitate on the parent/daughter ion that appears to give the best signal to noise ratio unless other factors indicate that another ion could give more accurate results. If interferences appear in the matrix, then two ions for each compound should be monitored, allowing the standard ratio to be compared to the sample ratio.

- Dilutions: If an analyte is detected at a level calculated to be above the calibration range, the sample will be diluted and reanalyzed.